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SEPTEMBER 1952
VOLUME 54 NUMBER 3

Published Monthly by
AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $8.00

TABLE OF CONTENTS FIRST PAGE

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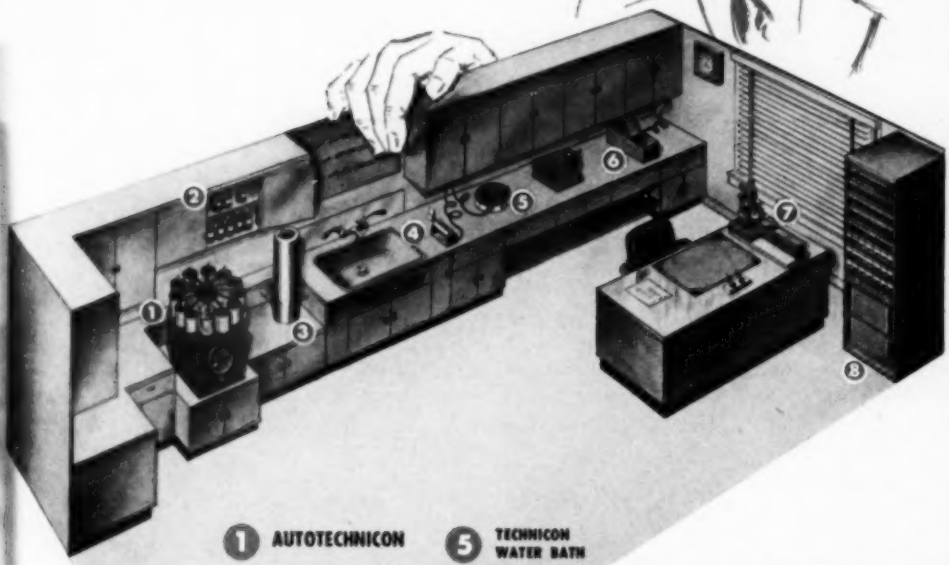

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CONTENTS

Original Articles

Anoxia as a Cause of Endocardial Fibroelastosis in Infancy PAGE
Frank R. Johnson, M.D., Chicago 237

Origin of Accessory Thyroid Tissue from Thymus IV in Adult Baboon
John H. Van Dyke, Ph.D., Philadelphia 248

Morphology and Pathogenesis of Biliary Cirrhosis
Eli Moschcowitz, M.D., New York 259

Kidney Lesions in Suckling Mice Following Group A Coxsackie Virus Infection
Roland Gädeke, M.D., Freiburg in Breisgau, Germany 276

Pattern of Mitotic Activity in Oral Epithelium of Rabbits
J. L. Henry, D.D.S., Ph.D.; J. Meyer, B.S.; J. P. Weinmann, M.D., and I. Schour, D.D.S., Ph.D., D.Sc., Chicago 281

Increasing Incidence of Arteriosclerotic Aortic Aneurysms
Rosario Maniglia, M.D., and John E. Gregory, M.D., Philadelphia 298

Case Reports

Bilateral Aplasia of the Lung
Peter E. Tuynman, M.D., and Lawrence William Gardner, M.D., Detroit 306

Metastatic Cystadenocarcinoma of Ovary Thirty-Three Years After Removal of Primary Growth
James B. Hutcheson, M.D., New York 314

Regular Departments

Books 319

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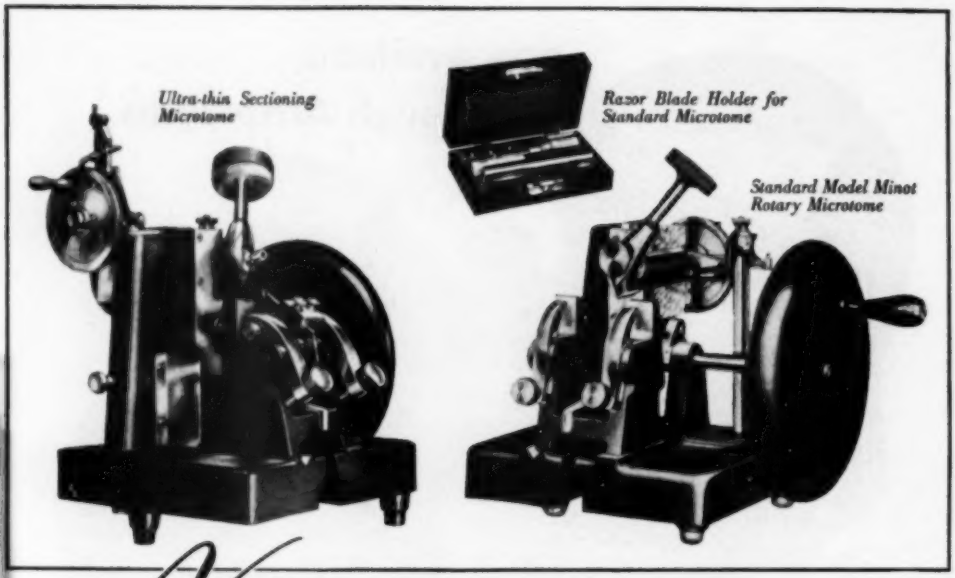

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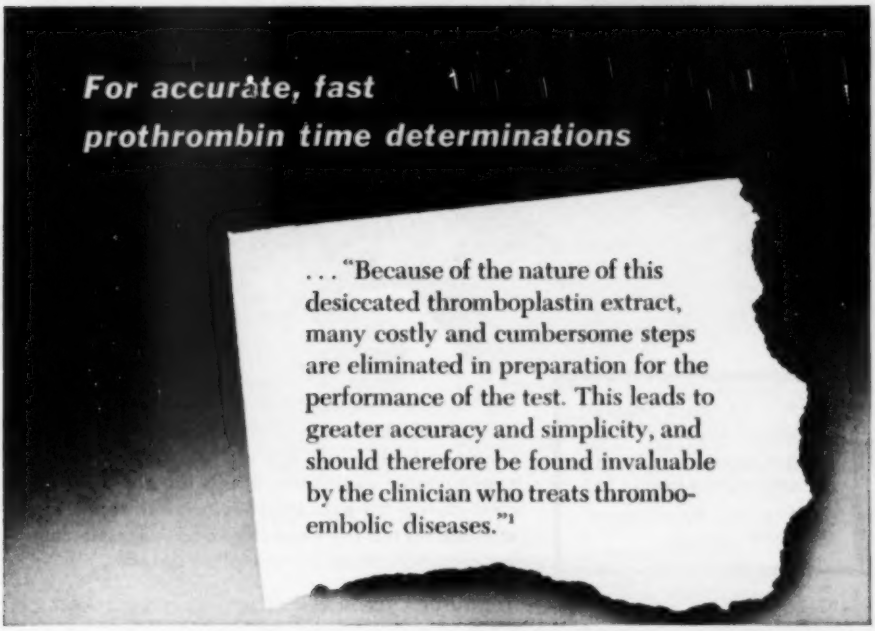
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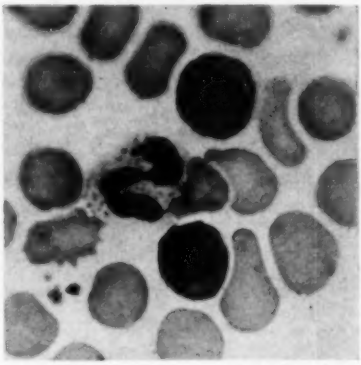

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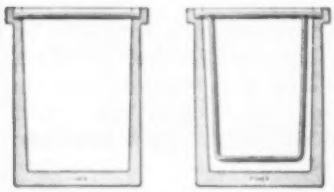

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ANOXIA AS A CAUSE OF ENDOCARDIAL FIBROELASTOSIS IN INFANCY

FRANK R. JOHNSON, M.D.
CHICAGO

ENDOCARDIAL fibroelastosis may be defined as either diffuse or focal thickening of the mural endocardium consisting predominantly of fibrous and elastic tissue. In the diffuse form the entire mural endocardium of one or more chambers of the heart is involved. Either the diffuse or the focal form may be accompanied by valvular stenosis.

The mechanism of development of diffuse endocardial fibroelastosis has been attributed by various authors to many causes. Recent opinions expressed in the literature appear to favor a congenital anomaly as the cause, but the type of the anomaly has not been agreed upon, and ideas range from heterotopic aortic tissue within the ventricular chamber [1] to simple hyperplasia of fibroelastic tissue.[2] In the past such factors as intrauterine infection,[3] toxins of maternal origin,[4] hypertrophy and hyperplasia of chromaffin tissue,[5] status thymicolymphaticus,[6] maternal hyper-

This work was partly supported by a Grant-in-Aid of the Chicago Heart Association.

From the Department of Pathology of The Children's Memorial Hospital and Northwestern University Medical School.

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tension,[7] a combination of mechanical stress and infection,[8] primary lung disease,[9] and ventricular dilatation with stretching of the endocardium [10] have been suggested as the cause. Of these many factors, only the ventricular dilatation with endocardial stretching has been found to have some factual basis, for Dewitski [11] was able to produce minimal endocardial fibroelastosis in rabbit hearts by repeated injections of epinephrine hydrochloride. Minimal endocardial fibroelastosis has also been found in the dilated left ventricles of patients dead of diphtheria.[12] This mechanism could, perhaps, also explain the minimal amount of endocardial fibroelastosis seen in the left ventricle in Fiedler's interstitial myocarditis and in glycogen-storage disease of the heart, and in the dilated right ventricle in idiopathic hypertrophy, pulmonary valvular stenosis and hypoplasia of the aorta. It is difficult, however, to explain severe cases of the disease on the basis of endocardial stretching, as the majority do not show ventricular dilatation.

MATERIAL AND METHODS

This investigation is based on an anatomical and histological study of 210 congenitally malformed hearts. Two control groups were used for purposes of comparison. The first consisted of 25 normal hearts from patients under 1 year of age, and the second of typical examples of glycogen-storage disease of the heart, Fiedler's interstitial myocarditis, healed myocardial infarction involving the endocardium, and severe left ventricular hypertrophy due to hypertension accompanying renal anomalies.

The hearts were opened as usual in the direction of blood flow and fixed in either Zenker's fluid or formalin. Blocks were taken from base to apex to include the left ventricle along the obtuse margin, the right ventricle along the acute margin, and the interventricular septum. In many instances the hearts were small enough so that this could be done in three vertically cut blocks, but in the case of larger hearts as many as six blocks were required to obtain adequate samples of these areas. In addition, blocks were cut from the right atrium, the left atrium, the junction of left ventricle and aorta, and the junction of right ventricle and pulmonary artery. Slides from each block were stained with hematoxylin-eosin, Verhoeff's elastic tissue stain, and phosphotungstic acid-hematoxylin.

RESULTS

Of the 210 congenitally malformed hearts, 23 had diffuse endocardial fibroelastosis and 187 had localized fibroelastic plaques. The pathologic anatomy of endocardial fibroelastosis is depicted in the illustrations and is too well known to justify further description. The localized fibroelastic plaques were usually discovered in places where increased strain might have occurred during life, such as the ventricular wall opposite an interventricular septal defect, the endocardium surrounding such a defect, or immediately below a severely stenotic valve. Localized plaques of endocardial fibroelastosis were occasionally seen in the 25 normal hearts, but they were much less common and of far less magnitude than those in the malformed group.

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The 23 hearts with severe diffuse endocardial fibroelastosis were associated with the congenital defects listed below:

1. Anomalous left coronary artery originating from the pulmonary artery	5 cases
2. Hypoplasia of the aorta	4 cases
3. Congenital mitral stenosis	1 case
4. Congenital mitral and aortic stenosis	1 case
5. Idiopathic hypertrophy	3 cases
6. Premature closure of the foramen ovale	2 cases?
7. Pulmonary atresia without interventricular septal defect	3 cases
8. Aortic atresia without interventricular septal defect	1 case
9. Pulmonary and tricuspid atresia with small interventricular septal defect	3 cases

Cursory examination of this tabulation gives the impression that the causes of the endocardial fibroelastosis were very diverse, and yet, close scrutiny discloses that there was one factor which was usually present in all cases. This was the factor of anoxia.

Anoxia of the endocardium, at this time of life, may be brought about in two ways. First, it may occur as a result of an inadequacy of the circulation. This is most frequently due to a congenitally aberrant coronary artery arising from the pulmonary artery instead of the aorta. Second, the endocardial anoxia may result from the presence of unoxygenated blood within the heart chambers during intrauterine life, most notably in premature closure of the foramen ovale or atresia of the pulmonary artery or aorta. It should be mentioned that a combination of these two factors may occasionally occur. It will now be appropriate to analyze the cases in this series and those in the literature to determine whether the findings are consistent with this hypothesis.

ABERRANT LEFT CORONARY ARTERY AND ENDOCARDIAL FIBROELASTOSIS

There are 36 cases of aberrant left coronary artery originating from the pulmonary artery [13] reported in the literature and there are 5 additional cases in this

13. Bland, E. F.; White, P. D., and Garland, J.: Congenital Anomalies of the Coronary Arteries, Am. Heart. J. **8**:787, 1933. Abrikossoff, A.: Aneurysma des linken Herzventrikels mit abnormer Abgangsstelle der linken Koronarterie von der Pulmonalis bei einem fünfmonatlichen Kinde, Arch. path. Anat. **203**:413, 1911. Heitzmann, O.: Drei seltene Fälle von Herzmissbildung, ibid. **223**:51, 1916. Kiyokawa, W.: Anomalie der linken Kranzarterie des Herzens und ihre Folgren, ibid. **241**:41, 1923. Scholte, A. T.: Über einen Fall von abnormer Abgangsstelle der linken Koronararterie aus der Pulmonalarterie, Zentralbl. allg. Path. **50**:183, 1930. Soloff, L. A.: Anomalous Coronary Arteries Arising from the Pulmonary Artery, Am. Heart J. **24**:118, 1942. Kockel, H.: Beitr. path. Anat. **94**:220, 1934. Rübberdt, H.: Abnormer Abgang der linken Kranzarterie aus der Lungenschlagader, Beitr. path. Anat. **98**:571, 1937. Dietrich, W.: Ursprung der vorderen Kranzarterie aus der Lungenschlagader mit ungewöhnlichen Veränderungen des Herzmuskels und der Gefässwände, Arch. path. Anat. **303**:436, 1939. Schley, J.: Frankfurt. Ztschr. Path. **32**:10, 1925. Mönckeberg, J. G.: Zentralb. Herz. u. Gefasskr. **6**:441, 1914. Eidlow, S., and McKenzie, E. R.: Anomalous Origin of the Left Coronary from the Pulmonary Artery, Am. Heart J. **32**:243, 1946. Proescher, F., and Baumann, F. W.: Abnormal Origin of the Left Coronary Artery with Extensive Cardiac Changes in a Child 13 Months of Age, J. Pediat. **25**:344, 1944. Sanes, S., and Kenny, F. E.: Anomalous Origin of the Left Coronary Artery from the Pulmonary Artery, Am. J. Dis. Child. **48**:113, 1934. Gasul, B. M., and Loeffler, E.: Anomalous Origin of the Left Coronary Artery from the Pulmonary Artery, Pediatrics **4**:498, 1949 (three usual cases). Carrington, G. R., and Krumbhaar, E. B.: So-Called Idiopathic Cardiac Hypertrophy in Infancy, Am. J. Dis. Child. **27**:449, 1924. Chown, B., and Schwalm, F. G.: Congenital Abnormality of the Heart, ibid. **52**:1427, 1936. Barnard, W. G.: Aneurysm of Left Ventricle Due to Left Coronary Artery Taking Origin from the Pulmonary Artery, J. Path. & Bact. **47**:345, 1938. Lyon, R.

(Footnote continued on next page)

series. Four of the patients whose cases are described in the literature lived to adult life, and free anastomoses between the two coronary arteries were demonstrated at postmortem examination. However, despite the anastomoses, endocardial fibroelastosis of the left ventricle was specifically mentioned in most of these reports. The remaining 32 patients and our 5 patients died in less than one year, and showed a uniform pathologic picture. There was moderately severe endocardial fibroelastosis of the left ventricle; in addition, there were degenerative changes of the left ventricular myocardium, including fibrosis, calcification, muscle degeneration, mild diffuse round cell infiltration, and in many cases aneurysmal dilatation of the apex. In hearts in which the coronary arteries were injected, anastomoses were not shown between the two coronary arteries. Thus in the reported cases of an aberrant left coronary artery arising from the pulmonary artery diffuse endocardial fibroelastosis not only has been constantly present but has been limited to the probable area of anoxia, namely, the left ventricle. In these cases, also, the underlying myocardium has exhibited signs of anoxic injury. Thus it seems highly probable that the fibroelastosis was a reaction to the anoxia. In these cases the lesion would correspond to the endocardial fibroelastosis which is seen in myocardial infarction in adults.

INTRAUTERINE ANOXIA AND ENDOCARDIAL FIBROELASTOSIS

Before considering the cases in which endocardial fibroelastosis may be attributed to the continued presence of unoxygenated blood within the ventricular or the atrial cavities, it will be helpful to discuss briefly the development of the coronary circulation and of the arterioluminal vessels. As pointed out by Lewis,[14] Grant,[15] Bennett,[16] and Patton,[17] the coronary circulation is relatively late in developing.

A.; Johansmann, R. J., and Dodd, K.: Anomalous Origin of the Left Coronary Artery, Am. J. Dis. Child. **72**:675, 1946 (two usual cases). Ruddock, J. C., and Stahley, C. C.: Anomalous Origin of the Left Coronary Artery, U. S. Navy M. Bull. **41**:175, 1943. White, N. K., and Edwards, J. E.: Anomalies of Coronary Arteries: Report of 4 Cases, Arch. Path. **45**:766, 1948. Kaunitz, P. E.: Origin of Left Coronary Artery from Pulmonary Artery: Review of the Literature and Report of 2 Cases, Am. Heart J. **33**:182, 1947. Linck, K.: Aneurysmatische Erweiterung der linken Herzkammer infolge Ursprungs der linken Kranzschlagader aus der Pulmonalis bei einem 8 Monate alten Mädchen, Arch. path. Anat. **297**:113, 1936. Bartsch, G. H., and Smekal, T.: Über den Ursprung eines Kranzgefässes aus der Lungenschlagader, Frankfurt. Ztschr. Path. **47**:256, 1935. Benešová, D.: Über einen ungewöhnlichen Ursprung der linken Kranzarterie aus der Lungenschlagader, ibid. **57**:265, 1942. Haferkorn, M.: Beitrag zu den Entwicklungsanomalien der Koronarateriен, Kinderärztl. Praxis **6**:13, 1935. Cazzaniga, A.: Anomala-Origine della arteria coronaria cordis sinistra dalla arteria polmonare, miocardite consecutiva: lieve trauma, morte immediata, Morgagni **57**:466, 1915. Heidloff, C. W. M.: Ein Fall von abnormen Ursprung der Arteria Coronaria Cordis sinistra Coronaria pulmonalis communis, Thesis, Leipzig, 1926. Orsós, F.: Über die Rolle der Coronargefässe beim Altern des Herzens, Beitr. path. Anat. **106**:1, 1941. Grayzel, D. M., and Tennant, R.: Congenital Atresia of Tricuspid Orifice and Anomalous Origins of the Coronary Arteries from Pulmonary Artery, Am. J. Path. **10**:791, 1934. Limbourg, M.: Über den Ursprung der Kranzarterien des Herzens aus der Arteria pulmonalis, Beitr. path. Anat. **100**:191, 1937.

14. Lewis, F. T.: The Question of Sinusoids, Anat. Anz. **25**:261, 1904.

15. Grant, R. T.: Development of the Cardiac Coronary Vessels in the Rabbit, Heart **13**: 26, 1926.

16. Bennett, H. S.: Development of the Blood Supply to the Heart in the Embryo Pig, Am. J. Anat. **60**:27, 1936.

17. Patton, B. M.: Micromoving Pictures and Electrocardiographic Records of Age Changes in Embryonic Heart Action, West. J. Surg. **52**:325, 1944.

Early in fetal life the coronary arteries supply only the epicardium and later grow inward by anastomosing loops to supply the myocardium and finally the endocardium. The arterioluminal sinusoids at first supply the entire heart; later, as the coronary circulation develops, they decrease in size, finally being replaced by, or included in, the latter circulation. However, the arterioluminal vessels probably function actively throughout fetal life. Wearn,[18] in experiments on normal adult dogs, showed that there exists a potential retrograde flow from the right ventricular chamber and that it functions actively whenever right ventricular pressure becomes equal to or greater than left ventricular pressure. These findings could be interpreted as related to coronary arterial pressure, which is dependent to a large extent upon the pressure in the left ventricle. Although not proved, it seems permissible to assume that some retrograde flow occurs whenever ventricular chamber pressure exceeds coronary arterial pressure, with blood flowing from the ventricular chamber through the endocardium into the subendothelial capillary plexus.

In fetal life the pressure of both ventricles is equal to, or greater than, the coronary arterial pressure and thus there is probably a continual flow of ventricular chamber blood through the endocardium.

The best example of a congenital anomaly in which unoxygenated blood is present within the left ventricle and atrium is premature closure of the foramen ovale. Normally oxygenated blood from the umbilical vein flows into the right atrium, where two parts enter the left atrium through the open foramen ovale, and one part enters the right ventricle.[19] In this way all chambers of the heart receive an adequate supply of oxygenated blood. However, if the foramen ovale closes during any part of fetal life, the left atrium and ventricle will receive no oxygenated blood. In these cases, then, one would expect to find endocardial fibroelastosis. This malformation is rare, but several cases have been reported. Craig [2c] described six hearts with closure of the foramen ovale or an intact atrial septum from patients who died three to nine days after birth, all of which showed marked diffuse endocardial fibroelastosis of the left atrium and ventricle. In addition, the left atrium and ventricle were small. Four other cases (Smith,[20] Lehman,[21] Bellett and Gouley,[22] and Vieussens [23]) with premature closure of the foramen ovale and a small left ventricle and atrium also showed severe endocardial fibroelastosis. An interesting case has been reported by Edwards and DuShane [24] in which there was a combination of premature closure of the foramen ovale, mitral atresia, and interventricular septal defect. Endocardial fibroelastosis was marked in the left atrium and absent in the left ventricle. This location of the endocardial fibroelastosis might have been predicted because of the fact that oxygenated blood reached the left ventricle

18. Wearn, J. T.: Observations on the Morphology and Functions of Some of the Components of the Coronary Circuit, Bull. Johns Hopkins Hosp. **68**:353, 1941.

19. Barcroft, J.: Fetal Circulation and Respiration, Physiol. Rev. **16**:103, 1936.

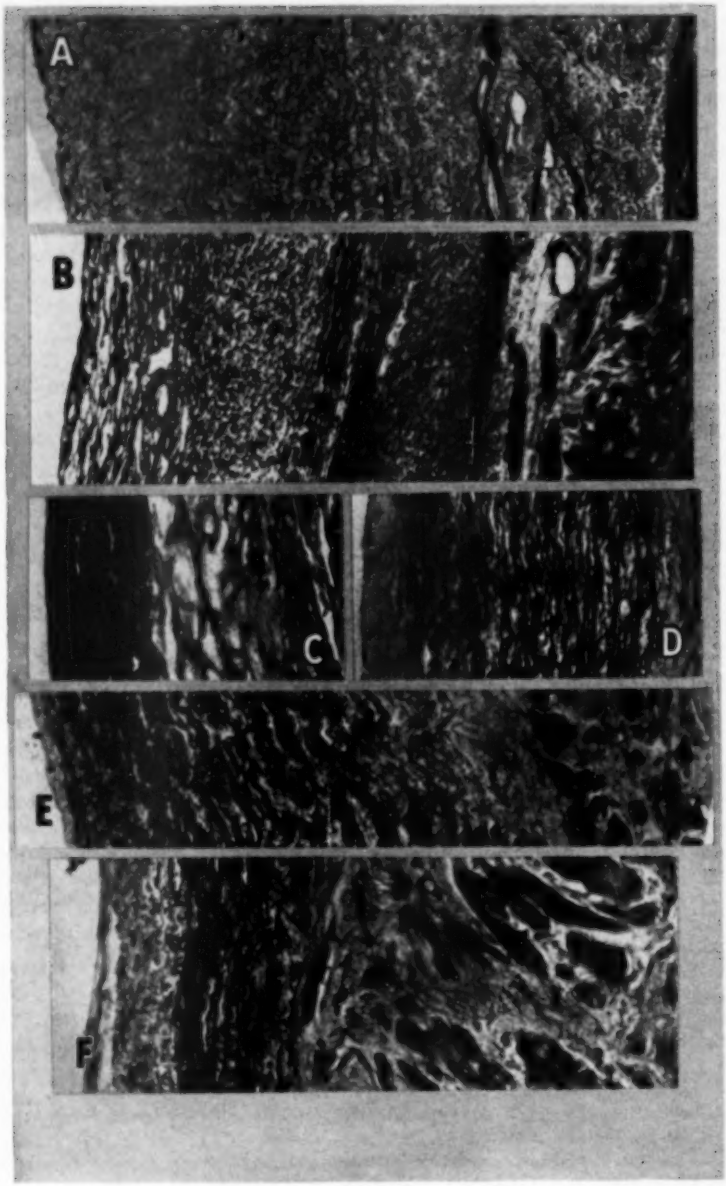
20. Smith, E.: Premature Occlusion of the Foramen Ovale, Tr. Path. Soc. London **39**:297, 1856.

21. Lehman, E.: Atresia of the Foramen Ovale, Am. J. Dis. Child. **33**:585, 1927.

22. Bellet, S., and Gouley, B. A.: Congenital Heart Disease with Multiple Cardiac Anomalies, Am. J. M. Sc. **183**:458, 1932.

23. Vieussens, R.: Traité nouveau de la structure et des causes du mouvement naturel du coeur, Ed. 1, Toulouse, J. Guillemette, 1715, p. 35.

24. Edwards, J. E., and DuShane, J. W.: Thoracic Vascular Anomalies, Arch. Path. **49**: 517, 1950.



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through the septal defect but could not enter the left atrium because of the mitral atresia. Also of interest is the case described by Vernon [25] in which the foramen ovale was closed but there was a large interventricular septal defect. No endocardial fibroelastosis was present. One additional case of premature closure of the foramen ovale is mentioned briefly by Patton,[26] but he did not describe the heart. In most cases of premature closure of the foramen ovale, the myocardium showed no severe degenerative changes, indicating that the reflux of oxygenated blood by way of the patent ductus arteriosus to the coronary arteries was sufficient to supply the myocardium. The cases of Osler,[27] Tait,[28] and Brenner [29] should be mentioned because they are usually referred to in the literature on this subject; however, the foramen ovale was patent in all cases, and the difference in size between the right and the left side of the heart was negligible. Such findings are hardly compatible with true premature closure of the foramen ovale.

In the present series there are no cases which fulfil the two essential criteria for the diagnosis of premature closure of the foramen ovale, first that the patient must die during neonatal life, in order to rule out the possibility of postnatal closure and, second, that the foramen ovale must be closed anatomically. There were, however, two patients who died seven weeks after birth in whom autopsy showed a complete absence of any remnant of the foramen ovale on the right atrial side of the interatrial septum and a left atrium and ventricle about half the expected size. Each had diffuse endocardial fibroelastosis in the left atrium and ventricle, degeneration of the myocardium immediately beneath the endocardium, and dilatation of the subendothelial sinusoids.

As all cases of premature closure of the foramen ovale were accompanied by diffuse left atrial and ventricular endocardial fibroelastosis, unless an interventricular

25. Vernon, H. H.: Two Cases of Malformations of the Heart, Tr. M. Chir. London, **39**:297, 1856.

26. Patton, B. M.: Developmental Defects at the Foramen Ovale, Am. J. Path. **14**:135, 1938.

27. Osler, W.: Cases of Cardiac Abnormalities, Montreal Gen. Hosp. Rep. **1**:177, 1880.

28. Tait, L.: A Case of General Dropsy in a Fetus, Tr. Obst. Soc. London **17**:307, 1876.

29. Brenner, M. C.: Premature Closure of the Foramen Ovale, Am. Heart J. **17**:437, 1939.

EXPLANATION OF FIGURE

A, hypoplasia of the aorta with endocardial fibroelastosis; phosphotungstic acid-hematoxylin stain; × 100. Note the extreme amount of endocardial thickening. In several areas within the endocardium (near the subendocardial border) one may see degenerating muscle fibers, completely surrounded by the fibroelastic tissue.

B, premature closure of the foramen ovale; Verhoeff's elastic tissue stain; × 100. Note the marked endocardial fibroelastosis with large and small interlacing elastic tissue bundles. The subendocardial sinusoidal dilatation and muscle degeneration may be seen.

C, idiopathic hypertrophy with slightly patent foramen ovale; Verhoeff's elastic tissue stain; × 250. The endocardial fibroelastosis is composed of thick elastic tissue fibers with some subendocardial muscle degeneration. Compare the thickness of the endocardium in this case with the idiopathic-hypertrophy variant below.

D, congenital mitral and aortic stenosis with closed foramen ovale (idiopathic-hypertrophy variant); Verhoeff's elastic tissue stain; × 250. Note the marked thickness of the endocardium, which is about halfway between the idiopathic hypertrophy and the premature closure. The elastic tissue fibers are in some areas coarse and in others quite delicate.

E, myocardial infarction involving the endocardium; Verhoeff's elastic tissue stain; × 250. Note the coarseness of the elastic tissue fibers and the fibrosis immediately beneath the endocardium. A small number of muscle fibers remain intact, forming a thin layer beneath the endocardium.

F, pulmonary atresia with intact interventricular septum; Verhoeff's elastic tissue stain; × 250. In this instance the subendocardial scarring is quite definite and penetrates deep into the muscle, between the muscle bundles. Within this scar tissue one may see the very delicate elastic tissue fibrils developing.

septal defect was present, it appears probable that the ventricular and atrial chamber anoxia produced by the closure of the foramen ovale is the most likely cause of the endocardial fibroelastosis.

STAGNATION ANOXIA DUE TO VALVULAR ATRESIAS

Pulmonary atresia without interventricular septal defect, aortic atresia without interventricular septal defect, and pulmonary and tricuspid atresia with tiny interventricular septal defect may all be discussed together, as the mechanism is probably similar in each. Blood would presumably enter the blind chamber, become trapped, and be unable to escape except in small amounts. This trapped blood, upon stagnation, would become relatively anoxic. However, in these cases, dilatation and forceful contraction of the myocardium against a relatively fixed volume of blood must also be considered as probably contributing to the production of the endocardial fibroelastosis. In this series there were three hearts with pulmonary atresia, all of which showed diffuse endocardial fibroelastosis of the right ventricle and degeneration and fibrosis of the underlying myocardium. Both ventricles were hypertrophied. There was also one heart with pure aortic atresia which showed similar changes limited to the left ventricle. In addition, there were three hearts with tricuspid and pulmonary atresia, in which, however, there was a small defect in the interventricular septum. The amount of endocardial fibroelastosis in these hearts was indirectly proportional to the size of the defect. Two other hearts with the same anatomic diagnosis, only with larger interventricular septal defects, showed no endocardial fibroelastosis.

In most of the cases of aortic atresia in the literature [30] there was far too severe a degree of endocardial fibroelastosis to be accounted for on the basis of stagnation anoxia alone, and these probably are better grouped with the cases of aortic hypoplasia. Roberts [31] reported an interesting case in which there were aortic atresia, severe endocardial fibroelastosis of the left atrium and ventricle, and a flap of tissue at the foramen ovale which effectively stopped blood from being shunted from the right to the left atrium despite any pressure differences.

DELAY IN PERFORATION OF THE SEPTUM PRIMUM AS A CAUSE OF ENDOCARDIAL ANOXIA

So far it has been shown that endocardial fibroelastosis occurred in hearts in which there were, in addition, malformations conducive to the development of anoxia in one or more of the heart chambers. There remain, however, several groups of cases in which objective clues to the mechanism of the development of the fibroelastosis are not readily apparent. These include the cases of aortic hypoplasia, congenital mitral or aortic stenosis, and idiopathic hypertrophy in which endocardial fibroelastosis was present. Nevertheless, it is possible to account for these cases on the basis of premature closure of the foramen ovale, and there are findings which suggest that the only essential difference is the time during intrauterine life at which the foramen ovale is closed.

As pointed out by Patton,[26] the foramen ovale is quite complex in its embryological formation. The septum primum first forms with the foramen primum, and

30. Wessen, H. R., and Beaver, D. C.: Congenital Atresia of Aortic Orifice, Stenosis of Ascending Aorta, Patent Foramen Ovale, Persistent Ductus Arteriosus, Ventricular Septum Entire, and Rudimentary Left Ventricle, J. Tech. Methods **14**:86, 1935.

31. Roberts, J. T.: A Case of Congenital Aortic Atresia, Am. Heart J. **12**:448, 1936.

as the former advances toward the fusing endocardial cushions, the latter gradually becomes smaller until only a pinpoint opening remains, which is finally closed by overgrowth of the half-moon-shaped septum secundum. As the foramen primum decreases in size, multiple perforations normally occur in the septum primum, at the place later to become the foramen ovale. Persistence of these perforations into neonatal life is one anatomic type of auricular septal defect. If, however, these perforations should be delayed, left atrial and left ventricular anoxia would result, causing the development of endocardial fibroelastosis. Once the process had begun, it would tend to become progressive throughout fetal life, owing to interference with the arterioluminal flow of blood resulting from the endocardial scarring. The left atrium and ventricle would remain small because the thickened endocardium would interfere with normal growth and because the increased pressure necessary to dilate these chambers would cause most of the blood to bypass the left side of the heart, thereby augmenting the process. The end result would be hypoplasia of the aorta with endocardial fibroelastosis. Indeed, as a general rule, the smaller the left side of the heart, the greater the endocardial fibroelastosis. This was true of the malformations of this type in this series and appears to be true of those reported in the literature.

PREMATURE FIBROUS TISSUE PROLIFERATION AT THE FORAMEN OVALE AS A CAUSE OF ENDOCARDIAL FIBROELASTOSIS

The cases of congenital mitral and aortic stenosis and of idiopathic hypertrophy with diffuse endocardial fibroelastosis may also be explained by the preceding hypothesis. In these cases the foramen ovale would probably be closed by a premature proliferation of fibrous tissue late in fetal life, for the foramen ovale closes by fibrous tissue proliferation, and not by differences in interatrial pressure (Patton [26]). That premature fibrous tissue proliferation may occur at the foramen ovale is indicated by the cases of Craig [26] and of MacGregor and McKendry.[32] In the former instance the heart was removed from an infant who was 1 month premature and who died on the first day of life. The foramen ovale was closed, but the left ventricle was dilated and hypertrophied with moderately severe endocardial fibroelastosis. Obviously, the foramen ovale had closed late in fetal life; otherwise the left ventricle and atrium would have been much smaller than normal, for it is not until the last six weeks to one month of fetal life that the pulmonary flow approaches or equals the flow through the umbilical vein,[25] and one would not, therefore, expect the left ventricle to be either of normal size or enlarged unless the closure took place in the latter part of pregnancy. The second case was that of an infant who died at birth

32. Benjamin, B., and Simon, M. A.: So-Called Congenital Idiopathic Hypertrophy of the Heart, Am. J. Dis. Child. **59**:842, 1940. Christie, A.: Normal Closing Time of the Foramen Ovale and the Ductus Arteriosus: An Anatomic and Statistical Study, ibid. **40**:323, 1930. Kugel, M. A., and Stoloff, E. G.: Dilatation and Hypertrophy of the Heart in Infants and Young Children, ibid. **45**:829, 1933. MacGregor, R. R., and McKendry, R.: Fetal Endocarditis, Canad. M. A. J. **50**:433, 1944. Philpot, N.: Congenital Atresia of the Aortic Ring, Ann. Int. Med. **2**:422, 1928. Stohr, G.: Malformations of the Heart of the Newborn, Arch. Path. **17**:311, 1934. Powers, G. F., and LeCompte, P. M.: Remarks on a Case of Congenital Idiopathic Hypertrophy of the Heart, J. Pediat. **13**:760, 1938. Weisman, S.: Congenital Idiopathic Cardiac Hypertrophy, Arch. Path. **33**:365, 1942. Winkler: Endokarditis foetalis, ein Beitrag zur Pathologie des angebornenen Herzfehlers, Verhandl. deutsch. path. Gesellsch. **15**:247, 1904.

following a Caesarean section at full term. In this instance the foramen ovale admitted only the tip of a small probe but was effectively closed by flaps of tissue on either side. The left atrium and ventricle were slightly smaller than normal, and endocardial fibroelastosis was moderately severe.

It is, of course, impossible to state the condition of the foramen ovale at birth in patients with congenital mitral and aortic stenosis or idiopathic hypertrophy, as the majority live to be several months of age, but it is significant that in the cases reported here, as well as in those described in the literature, autopsy revealed the following: 1. No defect of any magnitude has been noted in the interatrial or interventricular septum. The largest opening reported was barely capable of admitting a 0.2-cm. probe. 2. The percentage of patent foramina ovale was far below that even for adults. 3. Endocardial fibroelastosis is severest in the presence of congenital mitral or aortic stenosis, which invariably is associated with a closed foramen ovale, and least severe in the presence of idiopathic hypertrophy in which there is no valvular stenosis and an occasionally patent foramen ovale.

Endocardial fibroelastosis of the right ventricle in cases of congenital mitral and aortic stenosis and idiopathic hypertrophy is somewhat overemphasized in the literature. Although it is not infrequently present, the degree is never comparable to that present in the left ventricle and is usually of a more localized nature. When diffuse, it resembles the endocardial fibroelastosis of Fiedler's interstitial myocarditis or diphtheritic myocarditis, and could perhaps be accounted for on the basis of a rather acute dilatation, for when the foramen ovale closes, the right ventricle is forced to handle both the umbilical and the systemic circulation.

The size of the left atrium and ventricle and the degree of endocardial fibroelastosis will depend upon the time during intrauterine life when the foramen ovale closed. In the last group, the presence or the absence of an aperture, and, if present, its size and shape when the fibrous tissue proliferation had expanded itself, would also be an important variable.

In conclusion, therefore, there are probably three ways in which the foramen ovale may be closed:

1. Delay in perforation of the septum primum, causing the hypoplasias of the aorta with endocardial fibroelastosis.

2. Overgrowth of the septum secundum, causing the premature closures of the foramen ovale.

3. Premature fibrous tissue proliferation at the foramen ovale causing the congenital mitral and aortic stenoses and idiopathic hypertrophies.

MYOCARDIAL HYPERTROPHY

The factors leading to the development of myocardial hypertrophy in endocardial fibroelastosis are well presented by Gross,[2b] Weinberg and Himmelfarb,[2d] and Prior and Wyatt,[33] and the reader is referred to those articles.

SUMMARY AND CONCLUSIONS

A study has been made of 23 hearts with endocardial fibroelastosis and of the literature in an effort to determine the cause of this lesion. It has been found that

33. Prior, J. T., and Wyatt, T. C.: Endocardial Fibroelastosis: A Study of 8 Cases, Am. J. Path. **26**:969, 1950.

endocardial fibroelastosis always develops in hearts having associated malformations which could produce endocardial anoxia. These malformations usually involved (1) an anomaly of the coronary arteries in consequence of which unoxygenated blood was delivered to the endocardium, (2) premature closure of the foramen ovale, which prevented oxygenated blood from entering the left atrium and ventricle, or (3) valvular atresias in which stagnation anoxia developed. On the basis of this evidence it has been hypothesized that an important factor in the development of so-called congenital endocardial fibroelastosis is endocardial anoxia, just as it is in endocardial scarring in the adult and best exemplified in myocardial infarction due to obstruction of the coronary arteries. There may, of course, be other factors. No evidence, however, was obtained that the lesion is a true congenital anomaly or due to intrauterine infection.

ORIGIN OF ACCESSORY THYROID TISSUE FROM THYMUS IV IN ADULT BABOON

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FOR MANY years investigators have been concerned with the developmental fate of the derivatives of the embryonic pharynx in mammals, and particularly the so-called caudal pharyngeal complex. In most mammals, including both man and, apparently, the baboon, this is the fourth-pharyngeal-pouch complex, which gives rise to a parathyroid gland (IV), a "ventral diverticulum" (anlage of thymus IV), and an accessory outgrowth, known as the ultimobranchial body. The component of primary interest is the ultimobranchial body, which may have a greater importance in human thyroid physiology and/or pathology than formerly has been attributed to it.

While some investigators have made the claim that the caudal end of the embryonic pharynx (essentially the ultimobranchial body) in man possesses intrinsic thyroid-forming potentialities (Weller,[1] Norris [2]), there has been no real evidence forthcoming from studies in comparative anatomy or mammalian embryology to justify the interpretation that ultimobranchial tissue represents, per se, an inherent "lateral thyroid component" (Kingsbury [3]). Such evidence is also lacking in birds, in which this structure normally remains outside the thyroid gland (Dudley [4]).

When, because of abnormal development, the ultimobranchial body does not become enclosed within the growing mammalian thyroid gland, it may persist in the dorsolateral region of the neck as a relatively undifferentiated or cystic mass of cells; not infrequently it exhibits retrogressive characteristics (Watzka [5]). In man such cellular masses may give rise to new growths, known as "lateral aberrant thyroid tumors." These tumors have attracted much attention in the past, and their origin in some instances is questionable.

Dunhill,[6] however, expressed the belief that embryonic remnants of the "lateral anlagen"—the ultimobranchial bodies—being constitutionally inferior to the median

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This investigation is part of a study supported by a grant from the National Cancer Institute.

1. Weller, G. L.: Contrib. Embryol. (No. 141) **24**:93-138, 1933.
2. Norris, E. H.: Contrib. Embryol. (No. 159) **26**:247-294, 1937.
3. Kingsbury, B. F.: Am. J. Anat. **65**:333-359, 1939.
4. Dudley, J.: Am. J. Anat. **71**:65-97, 1942.
5. Watzka, M.: Ztschr. mikr.-anat. Forsch. **34**:485-533, 1933.
6. Dunhill, T. P.: Brit. J. Surg. **19**:83-113, 1931.

anlage, could become hyperplastic under the stimulation of ordinary physiological demands. He concluded that any excessive physiological demand upon them was followed by a papillary change (papillary cystadenoma). Cohn and Stewart [7] believed that the location of a papillary tumor either within or outside a lobe of the thyroid gland was dependent upon whether these embryonic cellular rests were separated from the lateral component before or after fusion with the main mass of the thyroid. The current, widely accepted concept, however, is that lateral aberrant thyroids (papillary tumors) represent either thyroid tumors that have split off from the main thyroid mass or metastatic lesions from primary growths within the thyroid gland proper (Warren and Feldman [8]). In this recent concept a most important possibility is ignored, namely, that the ultimobranchial bodies are the site of origin of so-called lateral aberrant thyroids and that primary tumors may appear simultaneously both in the thyroid and in the side of the neck as the result of "lateral" embryonic rests in each area.

Except for the recognition and surgical correction of human cervical tumors, there has been a relative lack of research on the postnatal thyroid gland of man, as well as that of higher monkeys. Studies dealing with components of the embryonic caudal pharyngeal complex (particularly the ultimobranchial body) have likewise omitted all consideration of the postnatal fate of these structures in mammals. Grafflin,[9] who has made the only report on the histology of the thyroid gland in the baboon, described one specimen. Apparently, he did not encounter an accessory thymic or thyroid mass in the vicinity of the thyroid in his adult male Chacma baboon (Papio porcarious), nor did he find these bodies in the adult male mountain gorilla (Gorilla gorilla beringei) which he also studied.[10] In both instances, however, Grafflin was concerned only with the thyroid gland proper.

The purpose of this paper, therefore, is to call attention to the spontaneous development of accessory thyroid tissue after birth, associated with a thymus IV outside the thyroid gland proper, in the baboon. My findings have more than academic interest. They help clarify the latent potentialities resident in certain portions of the caudal pharyngeal complex. They also substantiate the interpretations, derived from my previous studies, concerning the character of ultimobranchial tissue and its role in the production of thyroid tumors (Van Dyke [11]).

MATERIAL AND METHODS

The specimen studied was an old adult male baboon (Papio anubis; subgenus Choerapithecus). The animal was apparently in good health and weighed approximately 75 lb. (34 kg.). At autopsy, gross examination of the thyroid gland revealed a small oval body attached to the connective tissue capsule on the dorsomedial aspect of the left lobe of the thyroid gland. This structure was approximately 6 mm. long, 3 mm. wide, and 2.5 mm. thick, and was located just superficial to a rather inconspicuous vasculostromal hilus of the thyroid gland proper. The entire left thyroid lobe, together with this small appendage, was fixed in Helly's fluid and embedded in paraffin. Complete serial sections of this block of tissue were cut in the frontal plane (10 μ) and prepared for microscopic study by the usual staining procedures.

7. Cohn, L. C., and Stewart, G. A.: Arch. Surg. **40**:585-605, 1940.
8. Warren, S., and Feldman, J. D.: Surg., Gynec. & Obst. **88**:31-44, 1949.
9. Grafflin, A. L.: J. Morphol. **67**:471-476, 1940.
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11. Van Dyke, J. H.: Am. J. Anat. **76**:201-251, 1945.

OBSERVATIONS

Inspection of this oval appendage indicated an accessory thyroid lobule which was joined to an accessory thymic mass (thymus IV) by means of an epithelial cyst. These structures, together with the thyroid gland proper and parathyroid glands III and IV, are illustrated in Figure 1.

Thymus IV.—Reference to Figure 1 shows that approximately half this oval body was composed of thymus tissue. Except for one large, irregularly shaped medullary cyst, lined partly by stratified squamous epithelium and partly by simple columnar (or cuboidal) epithelium, this structure presented the typical picture of atrophic thymus tissue of advanced age. Manifestations of age involution were indicated by scattered calcium deposits and a thin cortex, containing isolated groups of fat cells. Although the medulla exhibited an abundance of fibrous connective tissue, a widespread cytoreticulum, possessing numerous mitotic figures, could be identified. Hassall's corpuscles were inconspicuous. Clusters of eosinophilic leucocytes and myelocytes, possibly derived from stem cells *in situ*, were also particularly noticeable in the medulla (Fig. 2, *a*). The connective tissue surrounding the whole mass of accessory thyroid and thymic tissue was rich in heterophilic leucocytes.

The medullary cyst extended from near the center of this thymic mass to its caudal end, where it was adjacent to the parenchyma of the accessory thyroid lobule (Figs. 1 and 5). The portion of the cyst intimately related to the medulla of the thymus was lined by simple columnar epithelium. It contained a pale basophilic colloid secretion of mottled character. The mottling was due apparently to the formation of clear (nonstaining) secretion droplets dispersed in the more basophilic substance of the cyst (Figs. 4, 5, and 6). In certain regions of the cyst these clear globules were so abundant as to impart a foam-like appearance. The connective tissue lying in apposition to the intramedullary portion of the cyst contained small clusters of cells with relatively clear cytoplasm and densely staining nuclei. Many of these cells formed small vesicles resembling young, aberrant thyroid follicles (Fig. 4, *a*; Fig. 7, *b*).

Accessory Thyroid Lobule.—This tissue lay within the same thin connective tissue capsule and occupied the upper portion of the accessory nodular mass illustrated in Figure 1. The region of the cyst associated with this accessory thyroid tissue was lined by a thick stratified squamous epithelium, which exhibited marked hyperplasia (Fig. 5, *c*; Fig. 7, *a*). An epitheliomatous character of the wall of this cyst was demonstrated by the "pearl" formations and the processes which radiated from its base. These manifestations are clearly shown in Figures 5, 6, and 7. These illustrations also represent the site of origin of certain gland-like cells from the epithelial cyst. Here, cellular extensions of the cyst wall were contiguous with small (young) vesicles of thyroid-like character (Figs. 5 and 6, *a* and Fig. 7, *b*). These vesicles were lined by densely staining columnar cells showing evidence of pyknotic nuclei, or partly by this kind of epithelium and large, clear cells with moderately staining nuclei, similar to the cells of typical thyroid parenchyma (Fig. 6, *a*). Many cells in small vesicles near the cyst possessed characteristics of degenerating epithelial cells (Figs. 5 and 6, *b*). Others appeared to have undergone a process of

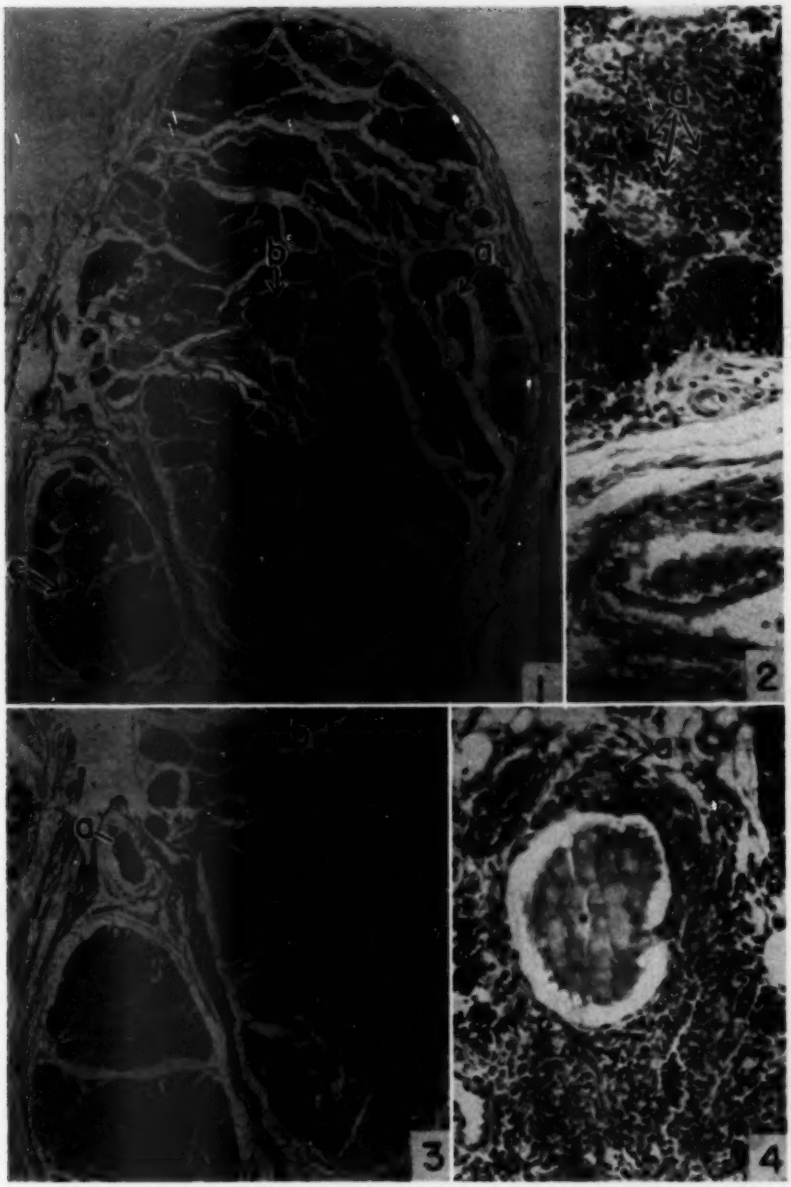

Fig. 1.—Thyroid gland containing parathyroid III (arrow at *a*) on the right and parathyroid IV (at *b*), near the center. Note appendage surrounded by its own connective tissue capsule and containing an accessory thymus (IV) joined to an accessory thyroid mass by an epithelial cyst (at *c*). Frontal section. × 15; hematoxylin and eosin.

Fig. 2.—Section of the medulla (above) and cortex of the atrophic thymus. Note clusters of eosinophiles (at *a*) and heterophilic leucocytes in the surrounding connective tissue capsule below. × 185; hematoxylin and eosin.

Fig. 3.—Note accessory thyroid lobe connected by a chain of vesicles to the main mass of thyroid tissue (at *a*) and parathyroid IV within the vasculostromal hilus (at *b*). This relationship substantiates the origin of the accessory thymus and thyroid tissue from fourth-pouch material. × 20; hematoxylin and eosin.

Fig. 4.—Intramedullary portion of epithelial cyst within the thymus IV. Note its cuboidal epithelium and the small thyroid-like vesicle derived from the wall of this cyst (at *a*). × 185; hematoxylin and eosin.

continued differentiation, resulting in normal-appearing thyroid cells and follicles. The more or less typical thyroid tissue represented by this accessory lobule can be seen in Figures 8 and 9.

Serial sections of the transitional area, extending from the accessory thymic body to the accessory thyroid lobule, revealed a gradual increase in the size of thyroid follicles. Small follicles were located near the cyst epithelium, while the larger vesicles (older) were more remotely situated. This finding gives one the impression that the accessory thyroid had only recently developed or was in the process of forming during postnatal life. Except for the differences noted above, the accessory thyroid gland was morphologically similar to the thyroid gland proper (Figs. 1, 9, 10, and 11). Indeed, a bridge or chain of thyroid follicles joined the two thyroid bodies at the site in which parathyroid gland (IV) had entered the main thyroid mass during embryonic development (Fig. 3, *a*).

Thyroid Gland Proper.—In general, the observations in this study conform with the description given by Grafflin.[2] Thyroid follicles were of various sizes and shapes. Large, thin-walled vesicles lined by a low cuboidal epithelium predominated. Attention is called to two conspicuous cell types. Typical colloid cells, with their hyperchromatic nuclei and homogeneous, acidophilic cytoplasm, were very numerous (Figs. 10 and 11, *a*). Cell counts indicated that these were more abundant in the main mass of thyroid tissue, although Grafflin did not call attention to colloid cells in his report. Typical principal cells, while occasionally encountered, were rare. Very noticeable, however, were large clear cells containing distinctly yellow or brown granules in both the accessory thyroid lobule and the thyroid gland proper (Figs. 9, 10, and 11.) Such granular cells apparently were not found in Grafflin's material. Their coloration has been regarded as the result of the reduction of potassium dichromate in Helly's fluid, which was used for fixation. They appear to represent an accumulation of lipofuscin-pigment granules associated with aging cells.

In Figures 6, 8, 9, 10, and 11, clear globules of secretion can be identified between the follicular epithelium and the acidophilic colloid. They may even occur in the midst of normal-appearing colloid and therefore cannot be confused with the so-called resorption vacuoles of some writers. Figure 11 clearly illustrates these globules adjacent to granular cells. In some cases they contain the extruded granules of cells from which the secretion was derived. It seems entirely possible that these droplets, derived presumably from modified principal cells, represent secretory products, of a character different from typical colloid, which have been dissolved away during the preparation of this material.

Here and there in the colloid, usually adjacent to follicular epithelium, small masses of dense basophilia were encountered. These are not uncommon in senile thyroid glands and have been interpreted as areas of calcification (Fig. 8).

Unlike certain other mammals (sheep, cat, and mouse), the baboon showed no evidence of ultimobranchial tissue which could be recognized as a discrete structure (cystic or otherwise) within the thyroid gland. It can only be assumed that the ultimobranchial body had undergone complete transformation into thyroid-like tissue within the thyroid during embryonic development, that it had completely degenerated (?), or that it had remained unincorporated (in part, at least), remaining in association with thymus IV outside the thyroid gland.

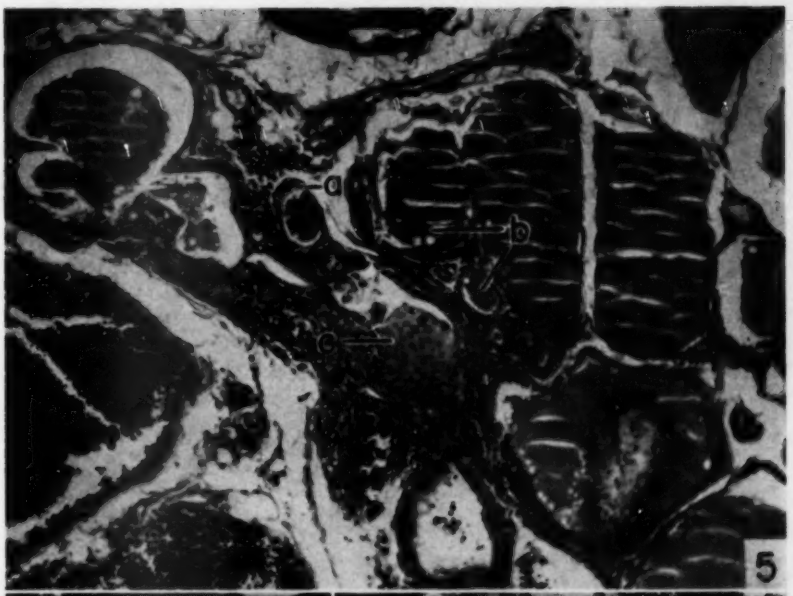

Fig. 5.—Transitional area between accessory thymic and thyroid tissue. The intramedullary portion of the cyst is on the left. Differentiating thyroid-like vesicles derived from cells produced by the cyst epithelium are indicated at *a*. Larger, more aberrant follicle formations, as indicated by their densely staining character, are illustrated at *b*. Hyperplastic stratified squamous epithelium of the cyst ("epithelial pearl") may be seen at *c*. × 135; hematoxylin and eosin.

Fig. 6.—Higher magnification of a portion of Figure 5. Note clear, more normal-appearing thyroid cells in follicle at *a*, as compared with other cells in the follicle. Note the clear globules of secretion associated with these cells. Such cells are regarded as arising by redifferentiation of preexisting, partially degenerated cells, similar to those illustrated at *b*. × 185; hematoxylin and eosin.

Fig. 7.—Section of cyst adjacent to the accessory thyroid tissue. Note its hyperplastic epithelium. Mitotic figures are indicated at *a*. Small vesicles on left (at *b*) are apparently recent derivatives of this cyst epithelium. × 140; hematoxylin and eosin.

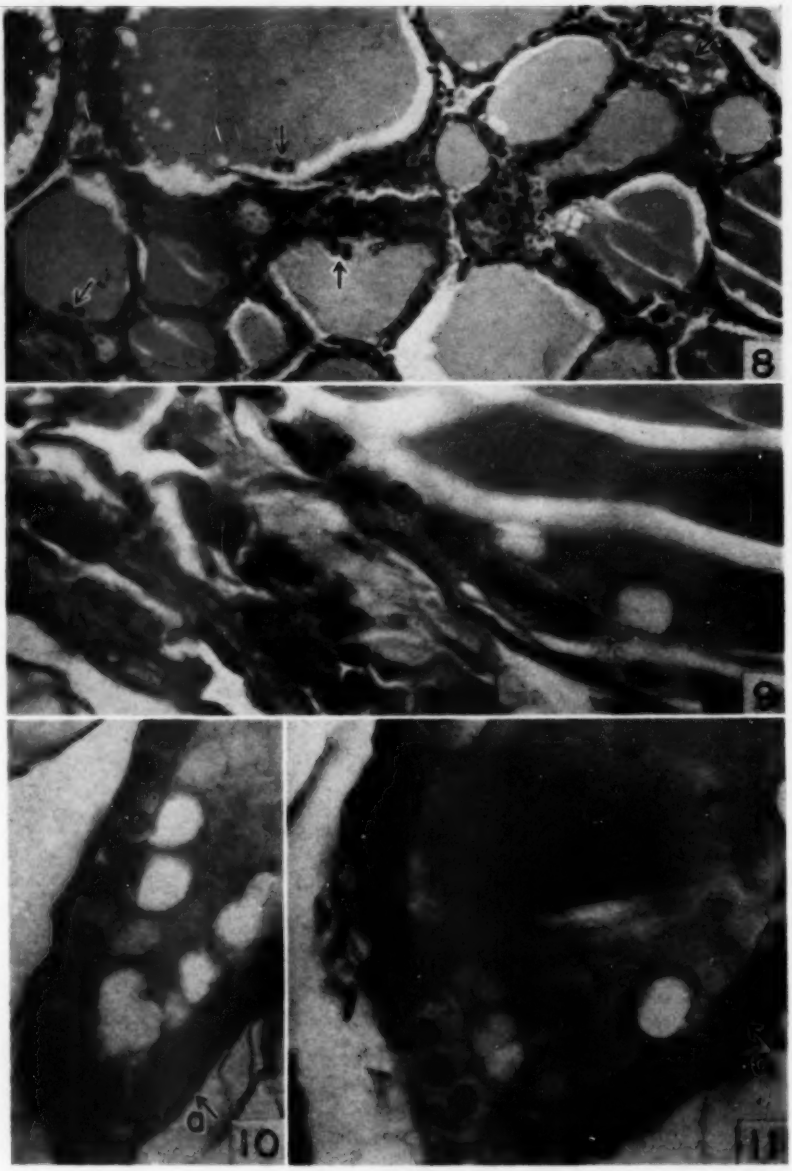

Fig. 8.—Section of accessory thyroid lobule, illustrating the histologic character of apparently normal thyroid parenchyma. Note droplets of basophilic secretion, or calcium deposition, in four follicles (arrows). Such basophilic foci were more numerous in the accessory thyroid tissue. × 200; hematoxylin and eosin.

Fig. 9.—Section of accessory thyroid tissue, showing a portion of a thyroid follicle on the right. Note the relatively clear, but highly granular, character of these epithelial cells. × 800; hematoxylin and eosin.

Fig. 10.—Portion of thyroid gland proper. Note large number of granular cells and the abundant serous droplets associated with them. Such droplets were more numerous in the main mass of thyroid tissue. A typical colloid cell is indicated at *a*. × 800; hematoxylin and eosin.

Fig. 11.—Portion of thyroid gland proper. Note the four clear, granular cells, each of which is associated with a serous droplet. Densely staining colloid cells can be identified (arrow at *a*). × 800; hematoxylin and eosin

Parathyroid glands III and IV were readily identified (Fig. 1, *a* and *b*). Parathyroid IV, the smaller of the two, was incorporated deep within a vasculostromal hilus at the dorsomedial aspect of the gland. Parathyroid III was larger, more superficial, and associated with the ventrolateral surface of the thyroid at the same level as parathyroid III. In keeping with the observations of Grafflin, we found no oxyphile cells. The glands were very vascular and compact.

COMMENT

The development of accessory thymus tissue (thymus IV) from a portion of the embryonic fourth-pharyngeal-pouch complex has already been reported for a number of common mammals (e. g., cat,[12] dog,[13] pig,[14], calf,[15] and opossum [16]). Studies in man indicate that a thymus IV may occur in approximately 43% of all fetuses examined.[17] To this list of animals in which at least an occasional thymus IV develops, the baboon should be added.

In some mammals, like the calf, the dog, and man, a thymus IV develops relatively much later than thymic tissue from the third pharyngeal pouch. In human specimens studied by me, a thymus IV was not identified before the age of 5½ months. It is quite possible, therefore, that accessory thymus tissue in this baboon arose during a comparable period of embryonic development. Although Kingsbury [18] believed that there was no reason to consider a thymus IV in mammals as arising solely from the fourth pouch, and not from the ultimobranchial body, it appears likely that the accessory thymic body in this baboon can be assigned to the fourth pouch proper and that the ultimobranchial body had been transformed into thyroid-like tissue.

Hyperplasias and neoplasias of thymus IV demand attention, infrequent as they may be, for their position on the dorsomedial surface of the thyroid facilitates encroachment upon the esophagus, as well as the trachea. A recognition of thymus IV, particularly in the simians, in which it has heretofore not been described, and in man, is of especial interest to clinicians and investigators interested in studies of hemopoiesis, exophthalmic goiter, calcium metabolism, and myasthenia gravis.

As far as I am aware, there are no authentic examples in which accessory thyroid tissue outside of the "median" thyroid gland has taken origin from a portion of the caudal pharyngeal complex. This is the only circumstance under which the ultimobranchial body, or any other part of the pharyngeal-pouch complex, could be regarded as an accessory source of thyroid tissue. My findings in the baboon, therefore, are quite unique. However, Godwin,[13] studying the development of thymus IV in the dog, observed thyroid-like follicles in the medulla of a thymus IV incorporated within the thyroid gland of a 34-day-old puppy. He apparently does not attribute the formation of these thyroid vesicles to any part of the fourth

12. Stewart, F. W.: Am. J. Anat. **24**:191-223, 1918.
13. Godwin, M. C.: Am. J. Anat. **64**:165-201, 1939.
14. Godwin, M. C.: Anat. Rec. **85**:229-243, 1943.
15. Kingsbury, B. F.: Am. J. Anat. **67**:393-435, 1940.
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17. Van Dyke, J. H.: Anat. Rec. **79**:179-209, 1941.
18. Kingsbury, B. F.: Am. J. Anat. **56**:445-479, 1935.

pharyngeal complex, for he states, (page 187): "In certain cases the thymus within the thyroid gland becomes so enlarged that it is more proper to say that thyroid is included within the thymus." In view of observations on the baboon, it appears more likely that such thyroid-like vesicles within the medulla of the dog thymus IV were derived from fourth-pouch material. Although not illustrated, similar vesicles were identified within the medulla of the baboon thymus IV.

The apparent fate of thyroid-incorporated ultimobranchial tissue has been elucidated from a morphological standpoint in a number of different mammals during embryonic development. Its involvement in thyroid physiology and/or pathology after birth, however, remains enigmatic. In young sheep the ultimobranchial body is usually represented in the thyroid gland by large, multiple cysts lined by stratified squamous epithelium. The cysts have been regarded as of congenital origin, due to the failure of the ultimobranchial body to fuse intimately with thyroid parenchyma during embryonic development. Moreover, the epithelium of these cysts frequently produces cords or clumps of gland-like cells that invade the neighboring thyroid tissue in a manner suggestive of a benign glandular neoplasm. Groups of these cells, most numerous in thyroids showing hyperplasia, form lobules that often differentiate into distinct thyroid-like follicles. In extremely hyperplastic, i. e., parenchymatous goiter, and in atrophic thyroid glands, these "new growths" may form conspicuous adenoma-like masses. In a recent review article, despite the functional "reactivity" of this aberrant thyroid tissue, I called attention to considerable evidence against the interpretation of the existence of a "lateral thyroid." [11]

It is also evident that this tissue is quite labile in the rat, being dependent upon the state of activity of the adjacent thyroid parenchyma. A condition of hyperplasia is conducive to its transformation into thyroid-like tissue; contrawise, an atrophic or relatively inactive state of the postnatal thyroid gland seems to foster the metaplastic transformation of this tissue into masses of epithelial cysts. In atrophic thyroids of old-age rats, such cysts can produce cells that ultimately form spontaneous cystadenomata (Van Dyke [19]). It has also been found that by the utilization of chronic vitamin A deficiency, metaplastic changes can be induced in the ultimobranchial-tissue component of the rat thyroid gland [20] and that tumors of this tissue can be produced experimentally by means of goitrogenic agents.[21]

Since transformation of ultimobranchial tissue into thyroid-like parenchyma during embryonic development in rats occurs at a time when the gland becomes functional,[22] it may act as a kind of growth center for relatively indifferent cells, which, after birth, can transform into thyroid tissue or tumors during periods of hyperactivity. It is interesting to speculate that a demand for more thyroid hormone, necessitated perhaps by adolescence, pregnancy, or other factors during the life of the individual, as suggested by Ward [23] to explain the existence of his "lateral aberrant thyroid tumors," might also apply in the case of this normal-appearing accessory

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22. Gorbman, A., and Evans, H. M.: Endocrinology **32**:113-115, 1943. Van Dyke.[19]
23. Ward, R.: Arch. Surg. **40**:606-645, 1940.

thyroid mass of the baboon. However, it does not explain why, in the face of much contrary evidence, ultimobranchial tissue does not more frequently produce essentially normal or tumorous thyroid tissue inside or outside the mammalian thyroid gland. Watzka,[5] who studied the unincorporated remnants of the ultimobranchial body in many mammals after birth, found nothing to support the view that this structure represents a "lateral thyroid gland." He concluded that cystic manifestations of ultimobranchial tissue (lined by various types of epithelium, and frequently composed of mucous-like acini) left outside the thyroid during embyronic development represented an expression of this tissue when not influenced by enclosure within the thyroid gland (Rogers[24]). Moreover, according to King and Pemberton,[25] there is no record of normal thyroid tissue having been found lateral to the thyroid gland during the course of several thousand radical dissections of the neck. However, since remnants of ultimobranchial tissue usually occur in relation to a thymus IV, accessory thyroid tissue, or, upon occasion, incipient neoplasms, when present, would be expected to lie on the dorsomedial aspect of the thyroid gland, rather than in the lateral side of the neck.

Under the circumstances, the concept that ultimobranchial tissue of mammals represents an inherent lateral thyroid component is not justified. But this does not preclude the possibility of this tissue forming an accessory thyroid lobe after prolonged stimulation by a hyperplastic thyroid gland. Without such stimulus, it might remain dormant indefinitely. Since it is known that thymic hyperplasias frequently accompany thyroid hyperplasias, a functional interrelationship between the thyroid and the thymus in mammals may be indicated. The thin-walled character of follicles, together with considerable colloid storage in the thyroid, of this baboon might suggest that the gland had been hyperplastic. Such a condition, bordering on colloid goiter, could be interpreted as involution following thyroid hyperplasia, as postulated by the Marine cycle.[26]

The suggestion that thymus tissue may occasionally produce thyroid tissue is also not remote, since experimental thyroid-like adenomata can be induced in the thymus of rats. This may explain the bizarre position of accessory thyroid tissue within the thoracic thymus of mammals, including man.[27] In view of the report by Chapman,[28] that administration of inorganic iodine to completely thyroidectomized animals increases the metabolic rate, and the findings of Soskin and Levine[29] that tissues other than the thyroid gland can manufacture thyroid hormone, the thymus, as well as the ultimobranchial body, takes on added significance. The thymus is thus suggested as an accessory source of thyroid hormone, and this possibility is now under investigation in our laboratory.

The position of parathyroid IV in the center of one lobe of the thyroid gland, strongly suggests that at least a portion of the ultimobranchial body had been carried into the thyroid gland of this baboon during embryonic development. However,

24. Rogers, W. M.: Am. J. Anat. **38**:349-377, 1927.
25. King, W. L. M., and Pemberton, J.: Surg., Gynec. & Obst. **74**:991-1001, 1942.
26. Marine, D.: J. A. M. A. **104**:2334-2341, 1935.
27. Van Dyke, J. H.: Anat. Rec. **112**:97-98, 1952.
28. Chapman, A.: Surg., Gynec. & Obst. **74**:483-486, 1944.
29. Soskin, S., and Levine, R.: Arch. Int. Med. **74**:375-383, 1944.

unless the accessory thyroid mass has arisen from thymus tissue, a part of the ultimobranchial body must have remained outside. On the basis of studies, both past and present, it appears that a portion of the ultimobranchial body, remaining outside the thyroid gland in this animal, had produced a large cyst lined essentially by stratified squamous epithelium. This cyst, as in the case of similar cysts within the thyroid of young sheep, is responsible for the growth of a single accessory thyroid lobule after birth, in response, probably, to thyroid hyperactivity.

SUMMARY

A typical accessory thyroid lobe, together with accessory thymus tissue (thymus IV), is described in the adult baboon. The evidence indicates that this accessory thyroid tissue has arisen after birth in conjunction with a thymus IV, probably from associated remnants of ultimobranchial tissue left outside the thyroid gland proper during embryonic development. The significance of the ultimobranchial body as a source of accessory thyroid tissue, as well as the "lateral aberrant thyroid tumor," is discussed on the basis of past observations and experimental ultimobranchial-tissue neoplasms which have been induced in the rat.

MORPHOLOGY AND PATHOGENESIS OF BILIARY CIRRHOSIS

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THERE are a number of problems in regard to biliary cirrhosis that await solution. 1. What are the relative roles of pure obstruction (cholestasis) and infection (cholangitis or cholangiolitis) in the production of the lesions? 2. When is biliary cirrhosis "primary" and when "secondary"? 3. Do the lesions caused by obstruction in the intrahepatic biliary channels differ from those caused by obstruction in the extrahepatic channels? 4. How far is the conventional classification of biliary cirrhosis valid? 5. Can a precise correlation be made between the clinical diagnosis and the morbid anatomy? 6. What is the effect of biliary cirrhosis on the vasculature of the liver and on the production of portal hypertension?

There are two reasons why these problems are still debated: 1. Only one of the backgrounds of biliary cirrhosis has been studied. 2. The problems have been approached from the static rather than the dynamic standpoint. It was therefore believed that a more integrated study might furnish answers to some of these problems, and so I have reviewed all the cases of biliary cirrhosis in the Mount Sinai Hospital in the past 25 years in which autopsy has been done. Particular emphasis was placed upon the study of the biology, in other words, the development of the lesion from the earliest stage to the end-stage. In this I was aided in a few cases by the findings in the lesions at biopsy, as compared with those at necropsy. Serial sections, in a number of instances, were invaluable in clarifying the derivation and destination of certain structures. All sections were stained with hematoxylin and eosin. In addition, Van Gieson's stain, the azan modification of the Mallory stain, the silver stain for reticulum fibers, and stains for glycogen were employed.

Altogether, 45 cases were collected. Of these, there were 6 cases of chronic intrahepatic cholangiolitic biliary cirrhosis; 12 of biliary cirrhosis following obstruction of the ducts of the liver by carcinoma of the head of the pancreas or of the hepatic ducts; 3 of biliary cirrhosis following cholangitis; 7 of obstruction of the common duct by stones; 8 of congenital atresia of the biliary tract, and 9 of obstruction of the common duct by stricture, metastatic carcinoma, and enlarged lymph nodes.

Data were obtained on the following factors: age; sex; weight of the liver; presence or absence of congestive splenomegaly; presence or absence of ascites and of esophageal varices; duration of the icterus; condition of the gall bladder and hepatic and common ducts, and cause of death.

From the laboratories, the Department of Pathology, the Mount Sinai Hospital.

MORPHOLOGY

First Stage of Biliary Cirrhosis.—The earliest lesion is best exemplified by the cases of obstruction of the common duct by carcinoma or stone, in which the duration of the jaundice is at the most a matter of a few weeks.

The portal spaces are infiltrated by cells, mostly lymphocytes, a smaller number of polymorphonuclear leucocytes, and a few plasma cells. This infiltration does not as a rule occupy the entire portal space but is limited mostly to the interlobular septa, particularly where the intercellular bile canaliculi join the smallest bile canals or cholangioles in the interlobular septa (Fig. 1). This is the area which Aschoff [1] termed the "Achilles heel." Occasionally suppurative cholangiolitis is associated, and the cholangioles contain polymorphonuclear leucocytes which also become the predominant cells in the portal spaces (Fig. 2*A* and *B*). Often this infiltrated area appears loose and edematous. The further evolution is represented by a progressive increase in the size of the portal space, due not only to the cellular spread but also to its fibroblastic differentiation, with capillarization and angiogenesis (Fig. 3*A* and *B*) such as I have described not only in Laennec's cirrhosis [2] but in all chronic inflammations.[3] In this interpretation the process represents a reversion to the embryonal

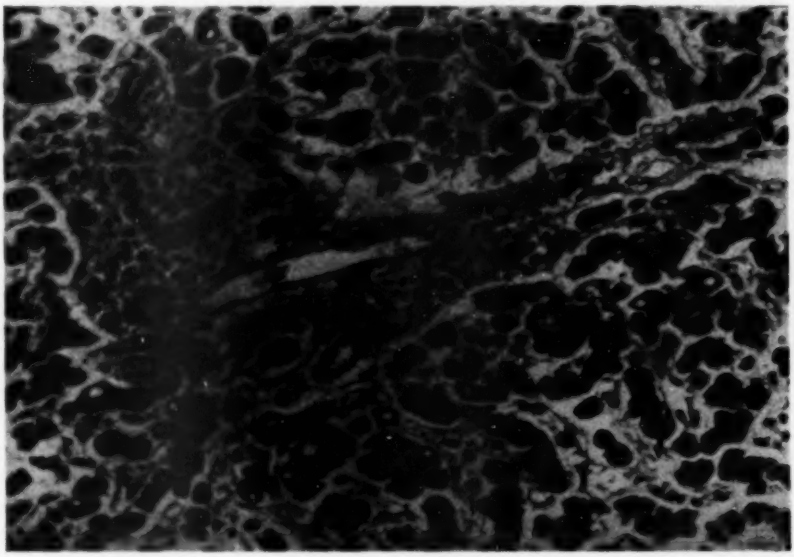

Fig. 1.—Lymphocytic infiltration surrounding cholangiole in interlobular septum; earliest phase of biliary cirrhosis or in a case of obstruction of common duct by stone. Duration of jaundice, four weeks. × 188.

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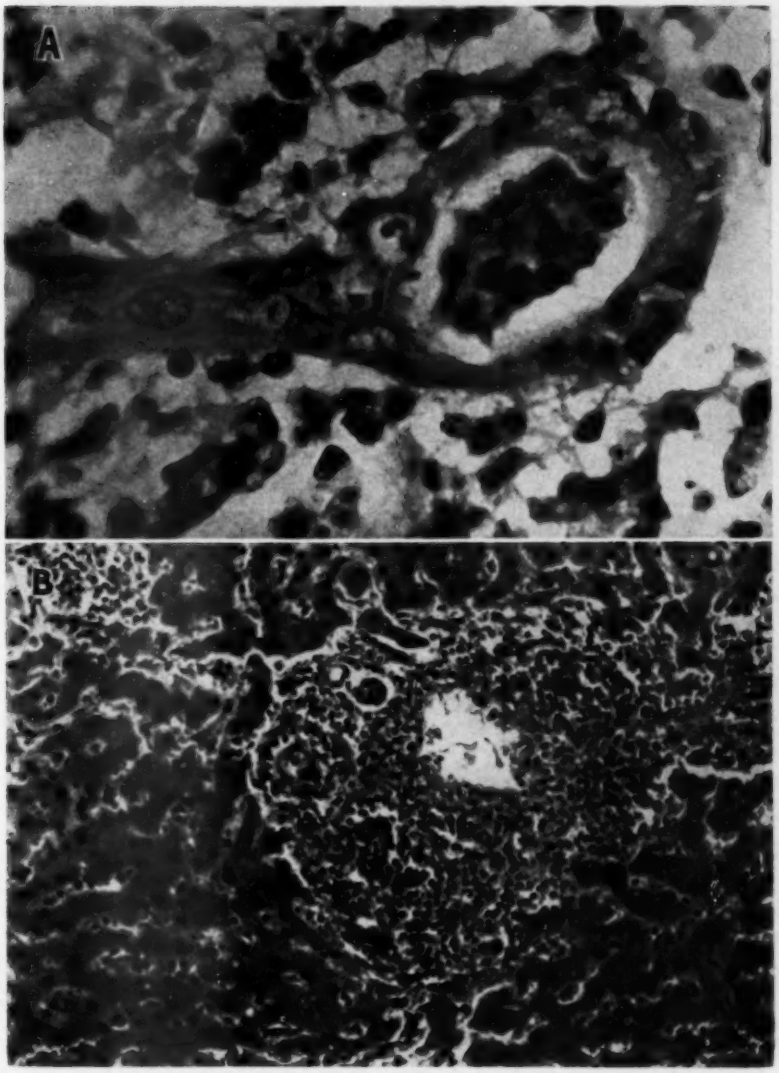

Fig. 2.—*A*, cholangiolitis with polymorphonuclear leucocytes within lumen in a case of obstruction of duct by carcinoma of pancreas. Duration of jaundice, four weeks. × 300.

B, early phase of cholangitis and cholangiolitis, with beginning portal cirrhosis; hematogenous (septic) origin. Duration of jaundice, seven days. × 188.

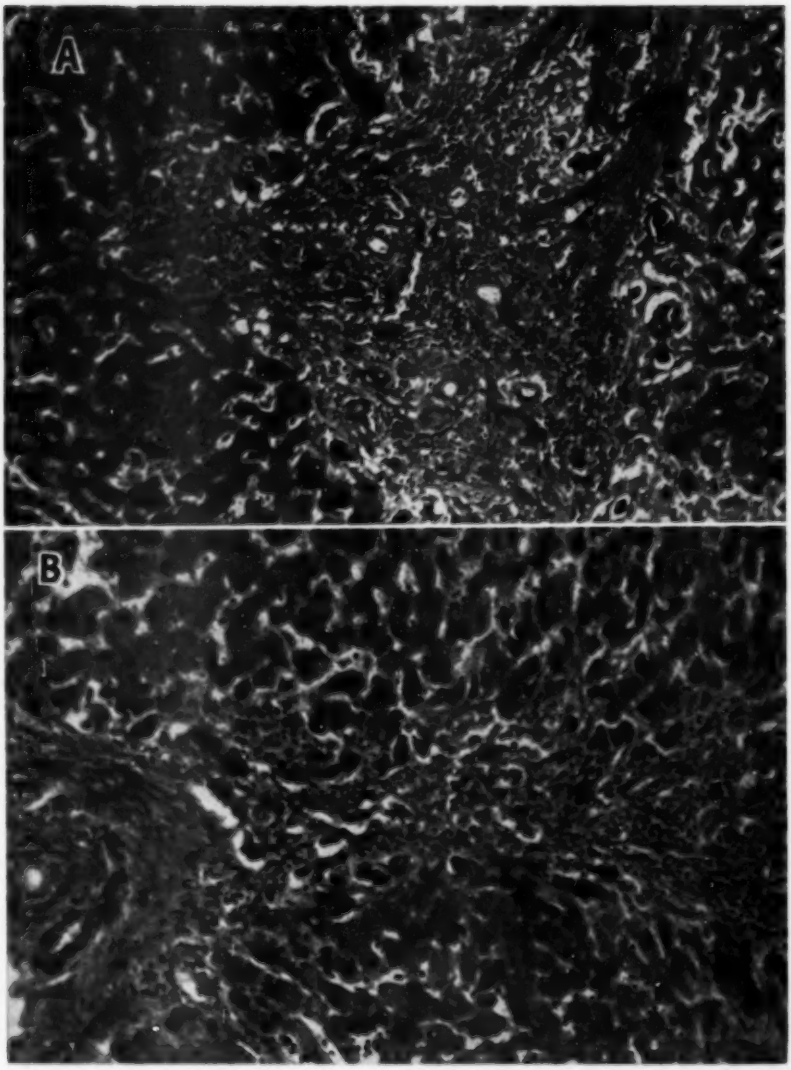

Fig. 3.—*A*, second phase of biliary cirrhosis, showing fibroblastic differentiation and capillarization, in a case of obstructive icterus due to stone in common duct. Duration of jaundice, six months. × 168.

B, second phase of biliary cirrhosis, showing fibroblastic differentiation and capillarization of interlobular septum, in a case of obstruction of common duct by stone. Duration of jaundice, one year. × 168.

mode of angiogenesis and thus follows the laws of Thoma.[4] In this phase the new areas of cellular differentiation may be viewed as a chronic granuloma which penetrates for short distances between the surrounding hepatic cords, with corresponding compression and atrophy of the cells. Interspersed in this granulomatous area are numerous newly formed bile canaliculi. In "catarrhal icterus," according to Axenfeld and Brass,[5] these canaliculi become visible between the 10th and the 15th day. These vary in number, being more numerous the more mature the granuloma; they are particularly abundant along the margins of the portal spaces. Transitions from hepatic cords are demonstrable, but whether the new bile canaliculi all have such an origin or whether some may arise from the interlobular bile ducts cannot be determined. In the early stages these bile canaliculi are solid; in the later stages they often develop a lumen, which sometimes contains biliary pigment. With stains for glycogen, the cells of these newly formed bile canaliculi are singularly free and can thus be differentiated from the normal biliary canals. Even in this stage biliary pigment is present in many of the intracellular biliary capillaries, especially along the margins of the portal spaces, around the central veins, and in some Kupffer cells. In exceptional instances small extravasations of bile occur in the parenchyma, which in the late stages are surrounded by a narrow zone of reactive fibrosis and occasionally by a giant cell of the foreign body type. Sometimes focal necroses in the parenchyma are seen, containing dead cells, shadow cells, and pyknotic nuclei; some of these foci contain bile pigment. Aside from a varying amount of fatty degeneration, the hepatic cord cells are usually well preserved. Despite the difference in time incidence between latent and manifest icterus, it was interesting to note a fairly close relation between the maturity of the granulomatous process and the duration of the jaundice. This will be more apparent when the later stages of biliary cirrhosis are described. In this stage, not all the portal spaces are affected by this fibroblastic differentiation, and in serial sections one level of a portal space may be affected while another is intact. In point of size all areas of the portal spaces are involved. Here and there the cholangiolitic process is manifested by the cuff-like cellular infiltration around some of the smaller bile ducts.

Grossly, the liver is only sightly enlarged and is bile-stained; the surface is smooth.

In this stage the normal architecture of the liver is fairly well preserved. The central and hepatic veins have their normal topography. The portal spaces are large but do not fuse. The interlobular septa are wider than normal and more prolonged. Occasionally a fibroblastic area containing newly formed bile canaliculi lies apparently isolated in the parenchyma, but on serial section these areas prove to be prolongations of the interlobular septa.

Second Stage of Biliary Cirrhosis.—This is exemplified in three cases: (1) a case of carcinoma at the junction of the right and the left hepatic duct in which jaundice had appeared 14 months previously; (2) a case of carcinoma of the head of the pancreas with a history of jaundice of 11 weeks' duration, and (3) a case of stricture of the common bile duct associated with a neurofibroma at the site of this stric-

4. Thoma, R.: Über die Strömung des Blutes in der Gefässbahn und die Spannung der Gefässwand, Beitr. path. Anat. **20**:67, and 92, 1919.

5. Axenfeld, H., and Brass, K.: Klinische und bioptische Untersuchungen über den sogenannten Icterus catarhalis, Frankfurt. Ztschr. Path. **57**:147, 1942.

ture, following a choledochotomy for stones 14 years previously, with recurrent attacks of jaundice.

In this stage the fibroblastic transformation is more mature and more extensive. Capillarization is more abundant and has proceeded to the point of formation of thin-walled venules. The interlobular fibroblastic septa extend deeper into the

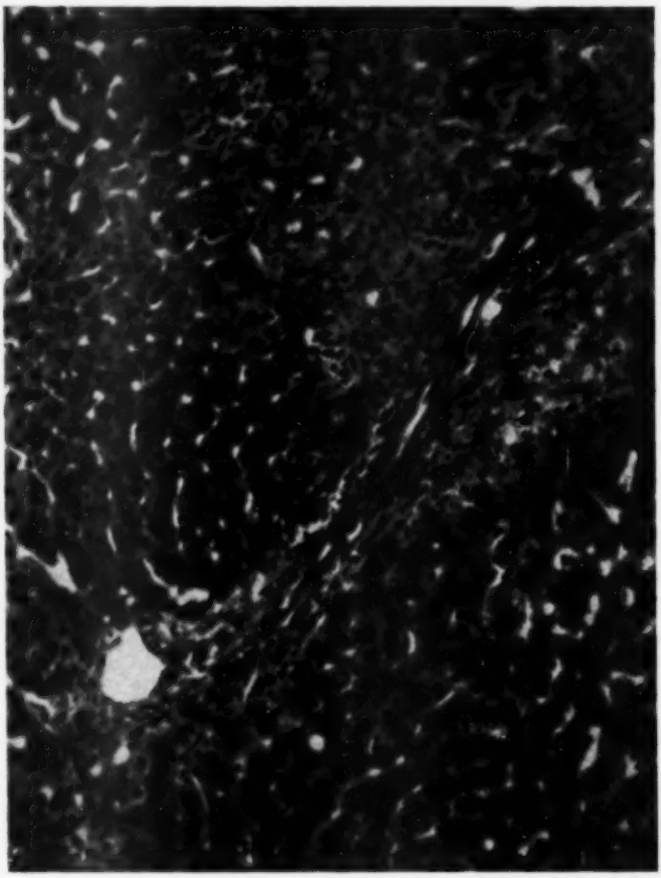

Fig. 4.—Fibroblastic interlobular strand with capillarization communicating with central vein in a case of chronic cholangiolitic biliary cirrhosis. Duration of jaundice, 16 months plus. × 167.

parenchyma and now fuse, forming pseudolobules and isolating smaller and larger groups of hepatic cords within the fibroblastic areas. Newly formed bile sprouts are more abundant, some of them having well-formed lumina, which contain bile casts. No trace remains of the cholangioles in the interlobular septa. The septa have been entirely replaced by the fibroblasts and collagen bundles. There are fibrous tissue bands between the portal spaces and the hepatic or central veins,

such as occur in Laennec cirrhosis (Fig. 4). In view of the fact that these bands contain capillaries and venules, one may conclude that these communications represent a reparative attempt to establish vascular channels between the portal vein and the hepatic veins, an internal Eck fistula, so to speak. The capsule of Glisson is now infiltrated with lymphocytes, and in the subcapsular space there is an irregu-

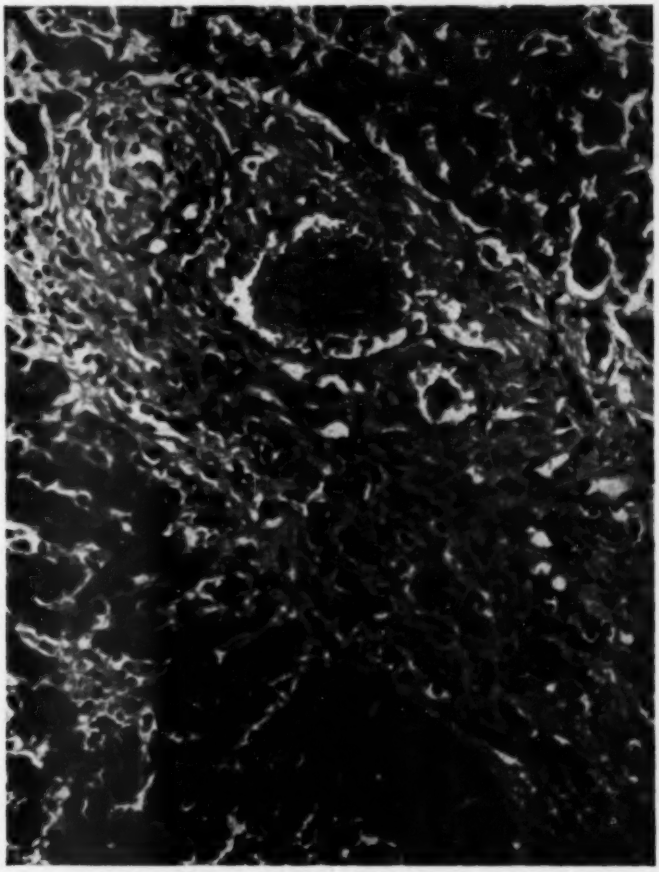

Fig. 5.—Portal space in which the bile duct has completely disappeared in a case of carcinoma of hepatic duct. Duration of jaundice, 14 months. × 183.

larly scalloped zone of fibroblastic tissue of the same maturity as that in the portal spaces, which is joined by bands to a neighboring portal space or to a hepatic or a central vein. Focal necroses are now rare.

The architecture of the liver is disorganized. It is difficult to recognize any but the largest portal spaces. The bile ducts of the smallest portal spaces have disappeared, leaving only the hepatic artery and portal vein (Fig. 5). The central vein

no longer bears its normal position in the lobule. Grossly, the organ is considerably enlarged, icteric, and nodular.

In two of the three cases, the spleen was enlarged, and while there was no outspoken congestive splenomegaly with sinus hyperplasia, both spleens showed fibrosis of the pulp cords, an evidence, as I have tried to show,[6] that hypertension of the portal circulation had developed.

Third Stage of Biliary Cirrhosis.—This stage was exemplified in 13 cases. Five were cases of extrahepatic obstruction, in three of which were strictures of the common bile duct dating back to a choledochotomy for stones four, five and six years previously; one was a case of impacted stone in the duct; of three years' duration, and in one there was a history of jaundice following an attack of cholelithiasis 15 months previously, proved by x-ray examination. At autopsy the bile duct was found to be dilated and to contain some exudate and debris. The gall bladder was atrophic. (It was surmised that a stone impacted in the papilla of Vater had passed.) In addition to these cases, there were eight cases of congenital atresia of the biliary tract.

This stage is characterized by a still greater maturity and by wider distribution of the fibroblastic process. While different phases of the granulomatous transformation of the organ are still visible, indicating a continuity of the process, the fibrotic transformation is predominant. Many of the smaller bile ducts in the portal spaces are surrounded by cuffs of lymphocytes. Their lumina are mostly free. The cords are fairly well preserved, but the parenchyma is invaded by intercommunicating bands of vascularized connective tissue, arising mostly from the interlobular septa, so that there is progressive increase of new tissue at the expense of the parenchyma. Many pseudolobuli are thus formed, and nests of cords are isolated in the fibroblastic tissue. The new connective tissue is now intralobular, interlobular, and perilobular. The normal architecture is completely disorganized, and, again, only the larger portal spaces can be recognized with certainty. The central veins are few and eccentrically situated. The hepatic veins are thickened, and many communicate with the portal spaces by vascularized connective tissue bands. The biliary ducts often show papillary-like folds, probably indicating a compensatory hyperplasia. This lesion was previously reported by MacMahon and Thannhauser[7] as "xanthomatous biliary cirrhosis." Biliary pigment is found intracellularly, within the newly formed bile canaliculi and within some of the Kupffer cells.

The capsule is now appreciably thickened, and the subcapsular zone is infiltrated with a more mature, granuloma-like tissue, which, as in the second stage, communicates by broad strands with adjacent portal spaces and hepatic veins (Fig. 6*A*).

Focal necroses are conspicuously absent. Evidences of regeneration of the hepatic cells in the form of mitoses are remarkably scarce. Grossly, the liver is still large, nodular, and bile-stained. I found it difficult to distinguish such a liver from one in the early stage of Laennec's cirrhosis. In order to determine whether there was any morphological difference between a case of the advanced form of biliary cirrhosis and a case of Laennec's cirrhosis in which jaundice appeared late,

6. Moschcowitz, E.: Pathogenesis of Splenomegaly in Hypertension of the portal Circulation: Congestive Splenomegaly, Medicine **27**:187, 1948.

7. MacMahon, H. E., and Thannhauser, S. J.: Xanthomatous Biliary Cirrhosis, Ann. Int. Med. **30**:121, 1949.

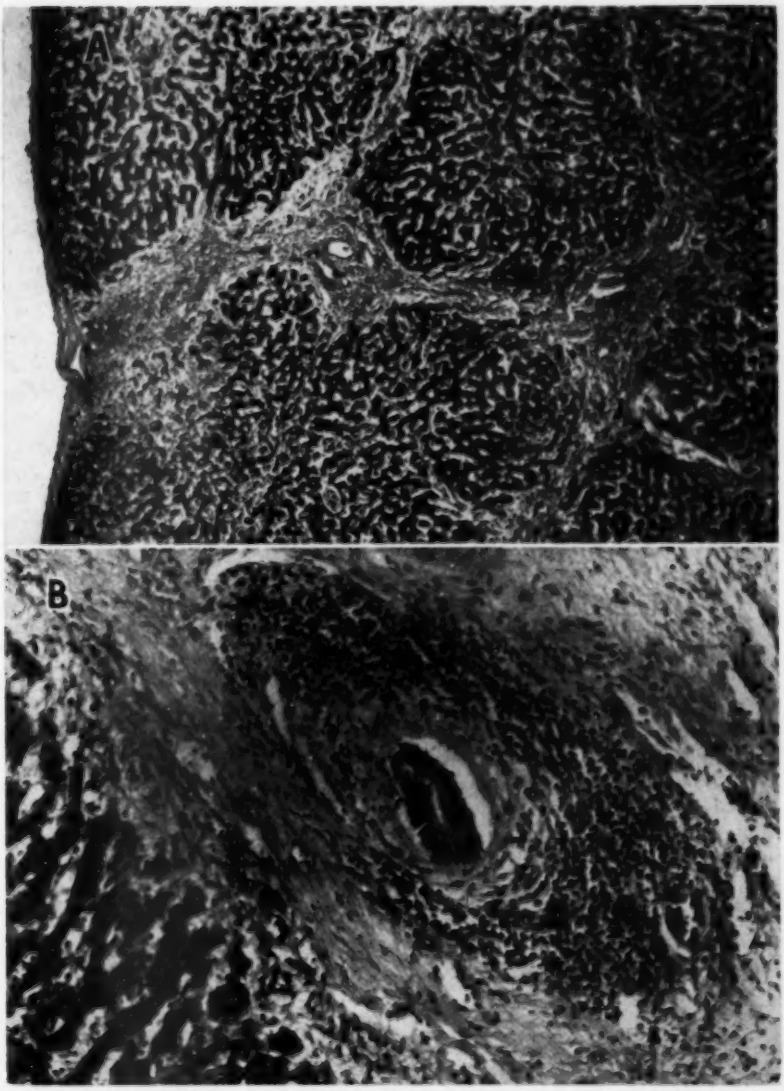

Fig. 6.—*A*, fibroblastic communication between capsule and hepatic vein and portal space in a case of stenosis of common duct following choledochotomy for stone. Duration of jaundice, three years. × 94.

B, lymphocytic infiltration of wall of small bile duct in a case of complete occlusion of common bile duct following choledochotomy. Duration of jaundice, three years. × 188.

I studied three cases of the latter. Except for the far greater number of newly formed bile canaliculi in biliary cirrhosis, I could not distinguish between the two. The only precise means of differentiation were clinical, namely, the history of alcoholism and the late appearance of jaundice in cases of Laennec's cirrhosis.

The morphological description thus far submitted agrees essentially with that of Lieber and Stewart,[8] who studied the changes in the liver in their cases of obstructive icterus due to pancreatic carcinoma. The connective-tissue content of the organ is so widespread that one would expect the development of a pronounced and prolonged hypertension of the portal circulation, and this indeed was found to occur, as manifested by the characteristic morphological changes of congestive splenomegaly.[8] These consist in a much enlarged, tough spleen, fibrosis of the pulp cords, sinus hyperplasia, *Aufsplitterung* (that is, with obscured borders replacing the normal sharp outlines between the fibrous septa and the splenic pulp), and *fibroadenie*. Congestive splenomegaly was found in three instances of the third stage in extrahepatic obstructions. In one case of extrahepatic obstruction due to an impacted stone of the common duct of three years' duration, the spleen weighed 460 gm. In the other two cases the cause of the extrahepatic obstruction was a stricture of the common duct dating from choledochotomies five and six years previously, respectively. In one case the spleen weighed 280 gm.; in the other the weight of the spleen was not mentioned in the protocol. Further evidence that hypertension of the portal circulation had arisen were the varicose esophageal veins. Of the eight cases of congenital atresia of the biliary passages, congestive splenomegaly was present in two, but esophageal varicosities were absent because, we believe, the hypertension did not last long enough, the ages of the patients being 6 weeks and 11 months, respectively. The observation that extrahepatic obstruction may cause congestive splenomegaly deserves emphasis, because, this aspect of biliary cirrhosis has not received attention in the literature. The only mention I could find were those made by Gibson and Robertson,[9] who described esophageal varices in one of their cases of stricture of the common duct, and by Klemperer,[10] who reported congestive splenomegaly. Above all this observation helps to interpret the pathogenesis of one of the most interesting and etiologically obscure forms of biliary cirrhosis, a form which has received various connotations, namely, "primary biliary cirrhosis," "intrahepatic obstructive biliary cirrhosis," "pericholangiolitic biliary cirrhosis," "chronic intrahepatic obliterating cholangitis,"[10] "xanthomatous biliary cirrhosis," and "primary intrahepatic cholangitis." Clinically, it has masked itself under the terms catarrhal icterus, hypertrophic biliary cirrhosis, cholangitis lenta, Hanot's (hypertrophic) cirrhosis, and even "Banti's disease." I shall designate this variety in accordance with the dominant morbid phenomena, namely, primary chronic cholangitic biliary cirrhosis.

Primary Chronic Cholangiolitic Biliary Cirrhosis.—Of the six cases, all but two were of females. The ages of the patients varied from 37 to 59, the mean age being 48. All the livers were enlarged; that is, they weighed more than 1,500 gm. and

8. Lieber, M. M., and Stewart, H. L.: Hepatic and Bile Duct Changes from Obstruction of Common Bile Duct Due to Pancreatic Carcinoma, Arch. Path. **17**:362, 1934.

9. Gibson, W. R., and Robertson, H. E.: So-Called Biliary Cirrhosis, Arch. Path. **28**:37, 1939.

10. Klemperer, P.: Chronic Intrahepatic Obliterating Cholangitis, J. Mt. Sinai Hosp. **4**:279, 1937.

were nodular and jaundiced. The duration of the jaundice varied from 15 months to 9 years.

Morphologically, the condition of these livers did not differ essentially from that described in the third stage of extrahepatic biliary obstruction. The granulomatous process again revealed that it was still continuing, but on the whole it was more mature, and inclined toward the collagenous and fibrous phases. Cholangiolitis was manifested by the fact that the walls of many of the smaller bile ducts, usually

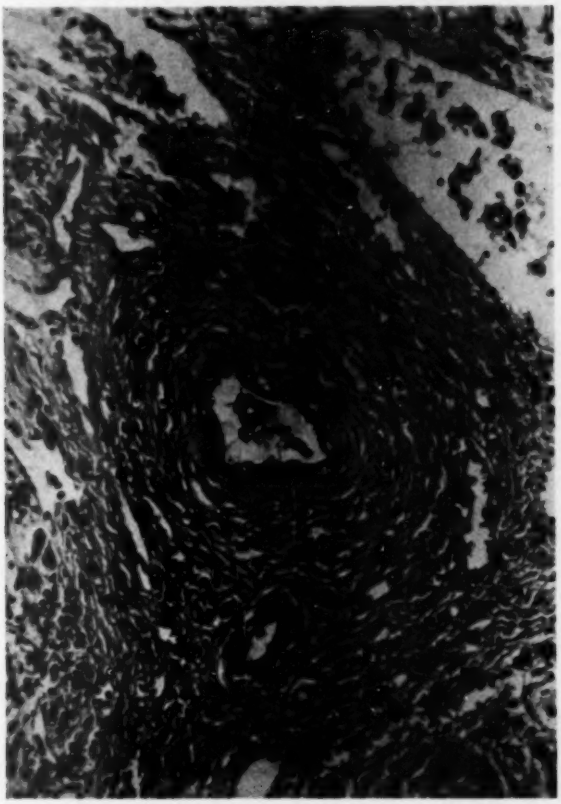

Fig. 7.—Stenosis of small bile duct in a portal space in a case of primary chronic cholangiolitic biliary cirrhosis. Duration of jaundice, three years. × 188.

in the area of the portal space where they become interseptal, were infiltrated with inflammatory cells (Fig. 6*B*). In serial sections one observed that the lining epithelium lost its normal appearance. The cells became irregular in outline, the nuclei varied in size and shape, and the cell bodies stained unevenly; eventually the bile duct became progressively smaller, and then disappeared within a focus of lymphocytes or fibroblastic tissue. In two cases the destiny of these interlobular ducts was revealed by a marked narrowing of the lumen by circumductal fibrosis

with flattening of the epithelium (Fig. 7), and occasionally by complete obliteration (Fig. 8), thus justifying the term suggested by Klemperer,[10] namely, "chronic intrahepatic obliterating cholangitis." At biopsy in one case I noted within some of these lymphocytic deposits giant cells surrounding amorphous transparent foreign bodies, which could not be identified. These bodies were not calcium. At

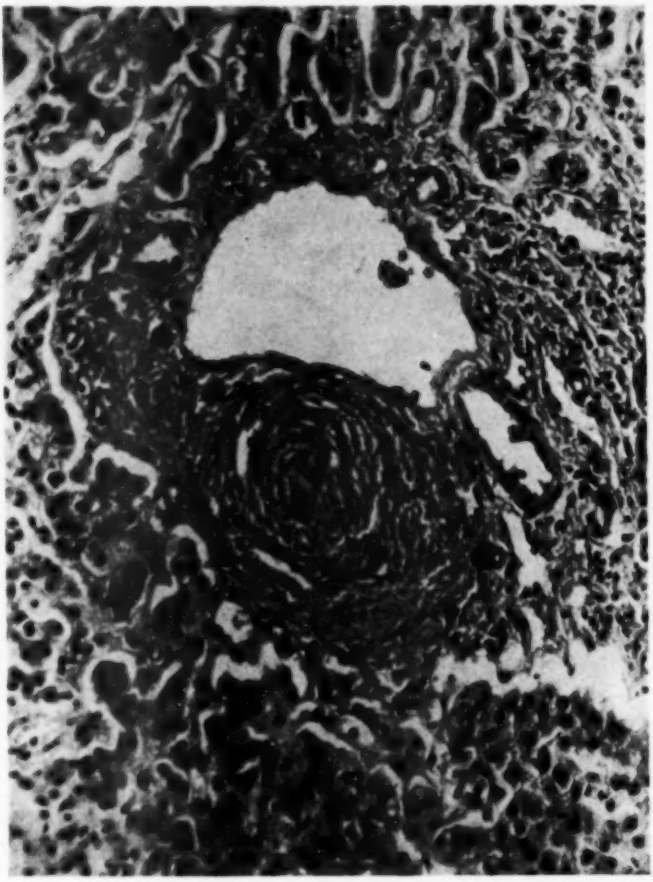

Fig. 8.—Complete obliteration of bile duct in a portal space by fibrosis in a case of chronic cholangiolitic biliary cirrhosis. Duration of jaundice, three years. × 209.

autopsy these foreign bodies were not observed. In three cases the papillary-like folds of the epithelium of the bile ducts, like those described in the final stage of extrahepatic obstruction, were noted. In four cases I was fortunate enough to study serially specimens obtained 14 months to four years before autopsy. In these cases biopsy showed a less mature phase. The lesions were not so widespread, and the architecture was less distorted. Focal necroses were absent.

In all six cases the spleen was considerably enlarged, the weights varying between 400 and 550 gm. In four cases there was pronounced congestive splenomegaly; in all these there were esophageal varices, and three patients died of gastrointestinal hemorrhage. One spleen showed intense congestion, and the condition of the other was described as infectious splenitis. No free bile in the parenchyma was noted. Apparently, this occurs only in cases in which the obstruction is complete. Xanthomas were not described in the present series, whether the obstructive cholangiolitis was extrahepatic or intrahepatic. I refer to this because it is now agreed [11] that the so-called xanthomatous biliary cirrhosis is not a sharply defined lesion and that the xanthomas may arise in any destructive hepatic disorder in which the serum lipids are greatly increased. The description given by MacMahon [11a] of such livers differs in no way from mine. The remarkable situation now confronts us that, although morphologically the ultimate phase of biliary cirrhosis sequential to prolonged extrahepatic obstruction is indistinguishable from that occurring in the chronic pericholangiolitic biliary cirrhosis, the mechanism of their production is entirely different. In the first, the cholangiolitis is the direct result of the obstruction; in the second, the cause of the cholangiolitis is unknown. Furthermore, in the second, that is, in chronic pericholangiolitic biliary cirrhosis, obstruction supervenes from the direct effect of the inflammatory activity. In other words, in one the obstruction is primary, and in the other it is secondary. Whether the secondary obstruction, in turn, intensifies the preexisting cholangiolitis is difficult to answer. An ascending infection can be excluded as the cause, since the biliary tract was entirely free, and with one exception, in which there was a history of diarrhea for some months prior to the onset of jaundice, the histories were entirely silent concerning a disorder that might have given rise to an ascending infection.

The alternative to this theory is a descending or hematogenous origin. A hematogenous bacterial origin of a cholangiolitic biliary cirrhosis has been frequently invoked,[12] but the number of valid cases are remarkably few. As Klemperer [10] insists, the cases must meet the following criteria: First, the extrahepatic biliary passages must be free from any evidence of past or present infection or any lesion that might possibly cause obstruction; second, there must be a focus of infection, either present at, or immediately preceding, the onset of jaundice. If, in addition, there is a positive blood culture, a hematogenous infection may be considered fairly certain. Of the reported cases, those that meet these criteria are cases of Fraenkel [13] and Siegmund; [12c] but of Siegmund's eight cases of supposed hematogenous origin, only two will pass the hazard of a strict critique. In my series, the cirrhosis in one case may be considered to be of hematogenous bacterial origin. A man, aged 25, had a laminectomy performed 10 days prior to consultation. Soon after the operation sepsis developed, with meningeal symptoms, and a blood culture showed

11. (*a*) MacMahon, H. E.: Biliary Xanthomatosis, Am. J. Path. **14**:527, 1948. (*b*) Ahrens, E. H.; Payne, M. A.; Kunkel, H. G.; Eisenmenger, W. J., and Blondheim, S. H.: Biliary Cirrhosis, Medicine **29**:299, 1950.

12. (*a*) Naunyn, B.: Über reine Cholangitis, Mitt. Grenz. Med. u. Chir. **29**:621, 1916-1917. (*b*) Schottmüller, N., in discussion in Aerztlicher Verein in Hamburg, München med. Wchnschr. **68**:1667, 1921. (*c*) Siegmund, H.: Selbständige intrahepatische Cholangitis, Beitr. path. Anat. **87**:425, 1931.

13. Fraenkel, E.: Über Paratyphuserkrankungen, besonders des Gallenapparats, München. Med. Wchnschr. **65**:523, 1918.

Micrococcus pyogenes var. aureus (Staphylococcus aureus). Four days later he became jaundiced. At postmortem examination there was a marked infiltration of the bile ducts with lymphocytes and polymorphonuclear leucocytes, extending at times deep into the portal spaces. The epithelium of some of the bile ducts was degenerated. There were slight fibroblastic differentiation and capillarization around the cholangioles, but no new bile sprouts. However, a hematogenous origin does not preclude an invasion by a toxin or a metallic poison. That arsenic may cause nonsuppurative cholangiolitis was proved by Hanger and Gutman [14] through biopsy in four cases after the administration of oxophenarsine hydrochloride (mapharsen®). Freis and Mater [15] and Loeper and Varay [16] each report a case. Similar findings have been reported after poisoning by manganese.[17] Toluenediamine has often been invoked as a cause of cholangiolitic cirrhosis, but the evidence is not confirmed by examination of tissue.

The histories in the present cases afford no suspicion of any invasion by a toxin or metal. Since there is evidence that vitamin deficiency, combined with the absence of a lipotropic factor, precedes the onset of Laennec's cirrhosis, I studied the histories for the abuse of alcohol or a nutritional disorder and found these factors absent.

PATHOGENESIS OF BILIARY CIRRHOSIS

Observers have debated the relative importance of obstruction and infection in the production of biliary cirrhosis. That pure obstruction without infection may cause biliary cirrhosis is shown in cases of infants with congenital atresias in various segments of the biliary tract. There is no evidence that these cases represent the late results of fetal cholangiolitis.[18] The observation that the cirrhosis in such cases is frequently associated with other malformations suggests strongly that these obstructions are the result of an error in development, due either to the failure of the primitive gut to form the hepatic ducts or to a faulty union of the two rudimentary portions of the hepatic and the common bile duct.

The results of experimental production of biliary cirrhosis by aseptic ligation of the common bile duct also suggest that obstruction without infection may be the only cause. While the results of experimental ligation of the common duct vary in different animals, by and large, the results parallel those I have described.[19] Focal

14. Hanger, F. M., Jr., and Gutman, A. B.: Postarsenaphenamine Jaundice, J. A. M. A. **115**:263, 1940.

15. Freis, E. D., and Mater, D. A.: Intrahepatic Obstructive Jaundice Following Mapharsen, with Development of a Spruelike Syndrome, J. A. M. A. **126**:892, 1944.

16. Loeper and Varay: Spleno-hepatitis icterogène et cholesterinogène, Bull. et mém. Soc. méd hôp. Paris **62**:525, 1940.

17. (*a*) Reddy, D. G., and Rao, S. V. R.: Blood and Bone Marrow in Pre-Cirrhotic Toxic Splenomegaly Due to Manganese, Indian J. M. Res. **32**:93, 1944. (*b*) Findlay, G. M.: Experimental Production of Biliary Cirrhosis by Salts of Manganese, Brit. J. Exper. Path. **5**:93, 1924. (*c*) Casamajor, L.: In Diseases of Occupation and Vocational Hygiene, Philadelphia, The Blakiston Company, 1916, p. 122.

18. Howard, C. P., and Wolbach, C. P.: Congenital Obliteration of the Bile Ducts, Arch. Int. Med. **8**:557, 1911.

19. (*a*) MacMahon, H. E., and Mallory, F. B.: Obstructive Cirrhosis, Am. J. Path. **5**:645, 1929. (*b*) Cameron, G. R., and Oakley, C. L.: Ligation of the Common Bile Duct, J. Path. & Bact. **35**:769, 1932. (*c*) MacMahon, H. E.; Lawrence, J. J., and Maddock, S. J.: Experimental Obstructive Cirrhosis, Am. J. Path. **5**:631, 1929. (*d*) Snell, A. N.; Greene, C. H., and Rowntree, L. G.: Further Studies in Experimental Obstructive Jaundices, Arch. Int. Med. **40**:471, 1927.

necroses appear in the early stages, only to disappear in the later ones. There is infiltration of inflammatory cells in the portal spaces, progressing to fibroblastic granulomas with capillarization. It is interesting in respect to my own observation that Rous and Larimore [20] and Snell and his co-workers [19d] reported the development of a large spleen and a collateral circulation in some of their long-term experiments.

Bile stasis is repeatedly invoked as the cause of the inflammatory change. However, the fact that a parenchyma containing abundant bile pigment is remarkably free from any reactive fibrosis, even over prolonged periods, would militate against this view. Moreover, it would hardly explain the exquisite limitation of the fibrosis to the portal spaces. The only occasion when bile may cause fibrosis is when it is extravasated into the parenchyma. It would, indeed, be rather remarkable for an organ with intact cells to react abnormally in an anatomical sense to a retained normal secretion. MacMahon and his co-workers [19c] and Lieber and Stewart [8] suggested that part of the mechanism is a rise of intraductal pressure. I incline to this view. That the biliary tract under normal circumstances sustains a pressure has been amply demonstrated.[21] There can be little doubt that the pressure rises if there is obstruction of the biliary tract. This interpretation parallels the changes that occur in hollow organs, for instance, the kidney and the pancreas, when the ureter or the pancreatic duct is obstructed over a long period.[22] Counseller and McIndoe [23] have shown that there is extensive dilatation of the biliary tract in obstructive jaundice owing to stone or carcinoma, which is sufficiently extensive to cause atrophy of the parenchyma and interference with the portal flow. From the analogy of the process to hydronephrosis, they term the sequence "hydrohepatosis." Whether the dilatation sufficiently compensates for the increased pressure remains to be determined. At all events, their demonstration proves that there has been a past or present intraductal hypertension. There is no evidence that some of the fibrosis may be due to the healing of the focal necrosis, since whenever intralobular isolated areas of fibrosis are found, serial sections show that they are always the widened, inflamed interlobular septa. The areas of necrosis probably heal by regeneration.[24]

A fundamental concept of inflammation is that it represents a reparative process. In discussing the pathogenesis of Laennec's cirrhosis, I submitted the suggestion that the large role played by capillarization and angiogenesis represents an attempt to compensate for the destruction of the capillary bed. This applies likewise to the pathogenesis of biliary cirrhosis. This compensation never fully meets the demands,

20. Rous, P., and Larimore, L. D.: The Biliary Factor in Liver Lesions, J. Exper. Med. **32**:249, 1920.

21. Potter, J. C., and Mann, F. C.: Pressure Changes in the Biliary Tract, Am. J. M. Sc. **171**:202, 1926. Winkelstein, A., and Aschner, P.: The Pressure Factors in the Biliary Duct System, ibid. **168**:813, 1924. Ivy, A. C., and Beigh, G. S.: Applied Physiology of the Extrahepatic Biliary Tract, J. A. M. A. **103**:1500, 1934. Doubilet, H., and Colp, R.: Resistance of the Sphincter of Oddi in the Human, Surg., Gynec. & Obst. **64**:622, 1937.

22. Weber, F. P.: On Biliary Cirrhosis With and Without Cholelithiasis, Tr. Path. Soc., London **54**:103, 1903.

23. Counseller, V. S., and McIndoe, A. H.: Dilatation of the Bile Ducts, Surg., Gynec. & Obst. **43**:729, 1926.

24. Zypkin, S. M.: Über die biliare Cirrhose, Arch. path. Anat. **262**:791, 1936.

and in consequence hypertension of the portal circuit ensues, which further induces congestive splenomegaly and sclerosis of the portal and splenic veins.

In addition, one may postulate that the extensive production of new bile ducts in biliary cirrhosis is a compensation for the obstruction and consequent obliteration of the intrahepatic bile ducts. In other words, just as the angiogenesis represents a compensatory attempt to restore the circulation, so these new bile ducts represent an attempt to restore the continuity between the main biliary passages within the portal spaces and the bile capillaries within the parenchyma.

CLINICAL IMPLICATIONS OF BILIARY CIRRHOSIS

It is apparent from the biological progression and the manifold backgrounds of the disease that a precise diagnosis of biliary cirrhosis in the early stages cannot be made during life except by biopsy. In this period the various tests for hepatic function are within the range of normal. In the later stages a presumptive diagnosis can be made if the duration of the icterus can be measured in terms of months, or preferably years, and if there are hepatic and splenic swelling, ascites, esophageal varices, and marked diminution in hepatic function. Whether the biliary cirrhosis is primary or secondary can only be proved by whether or not a cause is demonstrable. Even with all modern laboratory methods, resort to biopsy is the only means of conclusive proof.

I shall discuss only briefly the relation of biliary cirrhosis to two disorders with which it has often been considered synonymous, namely, "cholangitis lenta" and Hanot's cirrhosis. Cholangitis lenta was originally described by Schottmüller [12b] as part of a general bacterial infection caused usually by the viridans group of streptococci, the focus being in the biliary tract. Cases of this disease occur but rarely. Application of the term otherwise is not justified One can conceive that a cholangitis lenta may result in a biliary cirrhosis if it lasts long enough, but, as far as I am aware, no such case has been reported.

There is no doubt that the lesions of primary pericholangiolitic cirrhosis conform to many of the specifications set forth by Hanot [25] in his original publication. The fibrosis penetrates between the cords; there is pericholangiolitis, and there is no obstruction in the biliary tract. Unfortunately, Hanot described stones in the biliary tract in his first case, and the description of his second, fourth, and sixth cases suggests the diagnosis of chronic infectious hepatitis rather than of primary cholangiolitis. In the last analysis, therefore, Hanot's cirrhosis represents a syndrome, and not a specific morbid entity.

SUMMARY

Forty-five cases of biliary cirrhosis were studied. These can be grouped under two main headings: (1) biliary cirrhosis consequent to obstruction, and (2) biliary cirrhosis following a primary chronic cholangiolitis of unknown origin. The extrahepatic obstructions followed carcinoma of the head of the pancreas and the biliary ducts, cholelithiasis, congenital atresia of the biliary passages, acquired strictures of the common bile duct, and obstruction due to chronic cholangitis. The evolution of the lesions, from the earliest to the ultimate phases, in obstructions of extrahepatic origin are described. This is revealed in an inflammatory granulomatous process, progressive both in maturity and in extent, which originates around the

25. Hanot, V.: La cirrhosis hypertrophique avec ictère chronique, Paris, Rueff & Cie, 1892.

cholangioles. Thence it spreads into the contiguous areas of the portal spaces by a process of angiogenesis inherent in all granulation tissue. Capillarization occurs early. There is an abundant formation of new bile ducts. At first the granulomatous process is confined to the portal spaces. In the later phases prolongations of the interlobular septa fuse, forming pseudolobuli. Simultaneously, communications also arise between the portal spaces and the hepatic and central veins, giving rise to an internal Eck fistula. Subcapsular granulomatous lesions are now visible, which also communicate with the neighboring portal spaces and hepatic veins. In the ultimate phase, the normal architecture is completely distorted, and the smaller portal spaces and interlobular bile ducts are destroyed. The pathogenesis is viewed as a chronic cholangiolitis. Hypertension of the portal circulation sometimes develops, as proved by a congestive splenomegaly. There is a remarkable parallelism between the duration of the jaundice and the maturity of the lesions. In the second variety, namely, primary chronic intrahepatic cholangiolitic biliary cirrhosis, the morphological picture is identical with the ultimate phase of biliary cirrhosis due to extrahepatic obstruction. Occasionally, narrowing and complete obliteration of some of the smaller bile ducts occur, indicating widespread intrahepatic obstruction. Congestive splenomegaly was present in the majority of cases.

Pure obstructions, without the admixture of infection, may cause biliary cirrhosis. Experimental biliary cirrhosis simulates morphologically the cirrhosis which occurs in man. In the pathogenesis of obstructive biliary cirrhosis, increased tension within the biliary passages deserves consideration. A primary hematogenous cholangitis is exceedingly rare. Cholangitis lenta is a purely clinical designation and is by no means synonymous with biliary cirrhosis. Hanot's cirrhosis is a syndrome, and not a morbid entity. On morphological grounds only, the end-stage of biliary cirrhosis differs in no wise from that of Laennec's cirrhosis except in the presence of biliary pigment in the parenchyma and in the more abundant formation of new bile ducts.

KIDNEY LESIONS IN SUCKLING MICE FOLLOWING GROUP A COXSACKIE VIRUS INFECTION

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DEGENERATION of striated muscles is a characteristic of experimental Coxsackie virus infection.[1] The Group A strains induce widespread degeneration and creatinuria.[2] The present report is a description of associated, and presumably secondary, lesions of the kidneys of suckling mice infected with Group A strains and the relationship of the process to the crush syndrome.

METHODS AND MATERIALS

Albino mice were inoculated subcutaneously on the day following birth. Of the 32 animals which form the basis of the present report, 5 were inoculated with a Type 1 strain of Group A Coxsackie virus, 13 with a Type 2 strain, 5 with a Type 3 strain, and 9 with Group A strains other than the former but not further identified. One of the Type 2 strains, as well as the untyped ones, had been isolated by Vivell [3] from German children, by the inoculation of suckling albino mice with bacteriologically sterile fecal suspensions. All the strains used in the present work were demonstrated to be pathogenic only for immature mice and to induce in them extensive hyaline necrosis of the skeletal musculature. The strains used thus qualify as members of the Coxsackie group of viruses.[4] Since the muscle lesions were generalized and the interscapular fat pads and brains were not involved, the strains may be classified as Group A Coxsackie viruses.[5]

The tissues were fixed in 5% neutral formalin, embedded in paraffin, and stained with hematoxylin and eosin, the Weigert fibrin stain,[6] cresyl violet,[6] the Berlin and Turnbull blue methods,[7] the Lepehne technique for staining capillaries,[7] and the Heidenhain "azan" modification.[6]

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This study was aided by funds from the Notgemeinschaft der deutschen Wissenschaft.

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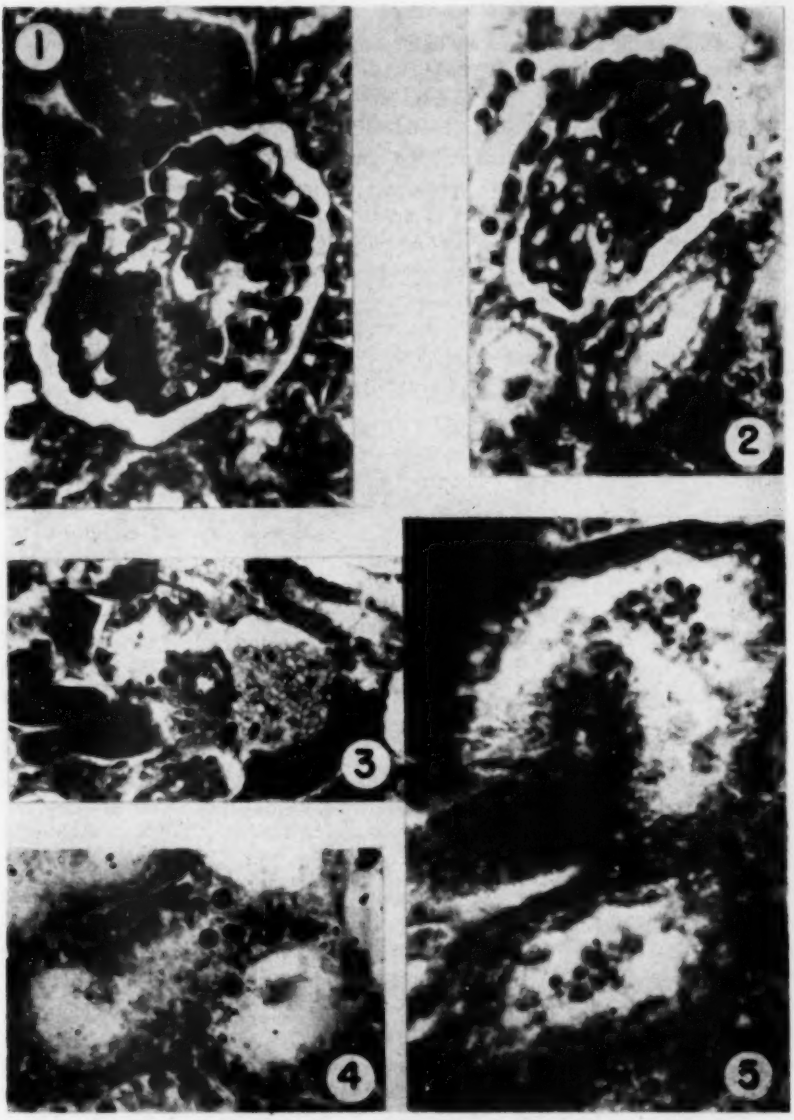

Fig. 1.—Swelling and hyalinization of glomerular endothelial cells; loss of nuclear staining of a few. Hematoxylin-eosin stain; × 950.

Fig. 2.—Hyperemia and collapse of glomerula loops. Hematoxylin-eosin stain; × 950.

Fig. 3.—Protein deposits in the lumina of tubuli contorti. Azan stain; × 450.

Fig. 4.—Intracpithelial protein droplets in tubular epithelial cells. Azan stain; × 950.

Fig. 5.—Protein clots and droplets in tubular lumina. Azan stain; × 950.

RESULTS

Lesions of the skeletal muscles were observed in each animal. The lesion consisted of fragmentation and swelling of the muscle fibers with loss of transverse and longitudinal striations, followed by homogeneous darkening and more intense eosinophilia of the sarcoplasm. In addition, basophilic iron and lipid-containing pigment were noted to accumulate in the fibers.[8] Frequently, fragmentation of the sarcoplasm was followed by the appearance of empty sarcolemma sheaths and the presence of phagocytosed droplets of material resembling the degenerated muscle protoplasm in the perivascular reticulum cells (Rouget cells). In later stages active regeneration was a prominent feature, the budding muscle cells being at times multinucleated. These lesions have been recognized as a feature of experimental Coxsackie virus infection from the beginning, although published reports do not record the emptying of the sarcolemma sheaths which has been so prominent in our material, and which may be related to the renal lesions.

Frequency of Kidney Lesions

		Type of Group A Coxsackie Virus with Which the Mice Were Infected			
		1	2	3	Other
No. of mice examined histologically		5	13	5	9
No. showing glomerular nephrosis		3	10	2	6
Tubular nephrosis	Epithelial lesions	5	13	5	9
	Casts	5	13	3	9
	Protein precipitates	2	13	0	5

The renal cortex was broadened and sharply demarcated from the medulla. In the subcapsular region the failure of metanephron and capillary structures to unite was observed in scattered foci. The deeper structures appeared fully developed, but the glomeruli were swollen and often distended their capsules. Swelling and hyaline changes in the capillary endothelium were common, as well as some loss of nuclear staining. The glomerular loops were either hyperemic or quite collapsed. The dilated capsules frequently contained protein precipitates. Swelling and desquamation of the endothelium of Bowman's capsule were seen at times.

The most striking changes were found in the tubular apparatus. The convoluted tubules were enlarged and the epithelium flattened. The cells stained dimly with hematoxylin and eosin, and in the azan preparations they were seen to contain numerous polychromatic (largely acidophilic) granules. Nuclear staining was poor. The free margins of the epithelium were indefinite and filled with protein floc or membranes. The proximal tubules contained greater amounts of azan-blue fragments, including droplets, lumps, and fine cloudy precipitates. In the ascending and descending loops of Henle were many such fragments, varying in size from roughly twice the dimensions of erythrocytes to the lower limits of visibility. Cylindrical casts were also found.

8. Gädeke, R.: Read at the Fifty-First Meeting of the Deutsche Gesellschaft für Kinderheilkunde, Heidelberg, Monatsschr. Kinderh. **99**:75, 1951.

The staining reactions of these masses were as follows: brownish-red in the hematoxylin-eosin stain, red in azan preparations, pale blue in the Weigert preparations, and Lepehne-reaction-positive in selected areas. In unstained preparations the color was dirty brown. The masses did not give the reactions of iron.

The frequency with which the various lesions occurred is shown in the Table. Photographs of representative lesions are shown in the Figure.

COMMENT

The lesion is a glomerular and resorptive nephrosis of the tubular structures, with a characteristic protein precipitate in the lumina essentially the same as that of the "crush kidney," the mechanism of which has been well discussed by Menkin.[9] The subsequent changes in capillary permeability and fluid retention result in oliguria and failure of adequate secretion of protein. In the present material the brevity of the disease process may have prevented the full development of the lesion. Similar pathologic alterations have been related to severe muscle destruction from many causes—accidents, war wounds, and others[10]—and are collectively referred to as "crush kidney." The loss of potassium and creatine noted by Gifford and Dalldorf[2] in mice infected with Coxsackie viruses also occurs in the crush syndrome of man.[11]

Creatinuria has also been observed in human cases of poliomyelitis,[12] which resembles Coxsackie virus infection in several respects. Whether the creatinuria of poliomyelitis is due to secondary degeneration of the paralyzed muscles or to direct involvement of the muscles by the virus is not known. The demonstration of poliomyelitis virus in the striated muscle in cases of fatal poliomyelitis[13] and of muscle

9. Menkin, V.: Newer Concepts of Inflammation, Springfield, Ill., Charles C Thomas, Publisher, 1950.

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11. Bywaters, E. G. L., and Stead, J. K.: Production of Renal Failure Following Injection of Solutions Containing Myohaemoglobin, Quart. J. Exper. Physiol. **33**:53-70, 1944.

12. Gros, W.: Der Kreatin-Kreatininstoffwechsel bei spinaler Kinderlähmung und seine Beeinflussung durch Glykokoll; Reaktion der Kreatinurie auf Glykokoll, Ztschr. klin. Med. **126**:152-160, 1933. Magers, E. J.: A Study of Certain Phases of Metabolism in Poliomyelitis, J. Biol. Chem. **105**:lvi (Sc. Proc.), 1934. Wang, E.: Clinical and Experimental Investigations on the Creatine Metabolism, Acta med. scandinav., Supp. 105, p. 1, 1939.

13. Jungeblut, C. W., and Stevens, M. A.: Attempts to Isolate Poliomyelitis Virus from the Paralyzed Muscle of Patients During the Acute Stage of the Disease, Am. J. Clin. Path. **20**:701-706, 1950.

lesions in experimental poliomyelitis and Columbia SK virus infection [14] may justify a search for renal lesions in these diseases. Myositis myoglobinuria and *Haffkrankheit*,[15] as well as similar diseases of lower animals, also deserve consideration.

SUMMARY

Nephrosis has been observed in suckling mice infected with various Group A Coxsackie viruses. The lesion is similar to the so-called crush kidney and may be due to the widespread destruction of striated musculature.

14. Verlinde, J. D., and Hofman, B.: Arch. Virusforschung, to be published. Rustigian, R., and Pappenheimer, A. M.: Myositis in Mice Following Intramuscular Injection of Viruses of the Mouse Encephalomyelitis Group and of Certain Other Neurotropic Viruses, J. Exper. Med. **89**:69, 1949.

15. Kaiserling, K.: Deutsche med. Wchnschr. **52**:316, 1926; Die histologische Untersuchung haffkranker Katzen, Deutsche med. Wchnschr. **58** p. 1934, 1932.

PATTERN OF MITOTIC ACTIVITY IN ORAL EPITHELIUM OF RABBITS

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THE PURPOSE of the present study was to investigate the pattern of mitotic activity by which the epithelium of the oral cavity of the rabbit is maintained.

Although it has been claimed that very rapid desquamation of superficial cells and compensatory mitotic activity in the deeper layers of the epithelium occur everywhere in the oral cavity, particularly in those regions on which great demands are made,[1] no quantitative studies of epithelial regeneration in the oral cavity are on record. Such studies have been carried out in the epithelium of the skin,[2] the urinary bladder,[3] and the intestinal tract,[4] and have shown widely different patterns of mitotic activity. It was therefore thought interesting to investigate and compare the epithelium of the oral mucosa with these other types of epithelia.

MATERIAL AND METHODS

This investigation was based upon a histologic study of the oral epithelium of 54 New Zealand white rabbits. The animals were usually obtained in groups of five littermates. They were fed

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This article is part of a dissertation submitted by Dr. Henry in partial fulfillment of the requirements for the degree of doctor of philosophy in anatomy at the University of Illinois.

The investigation was carried out under contract W49-007 MD-496 with the Medical Research and Development Board, Office of the Surgeon General of the United States Army.

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Dixie rabbit pellets,[5] supplemented by carrots and carrot tops twice weekly. At the time of observation, the rabbits were 3½ months old and had a mean weight of 1,950 ± 136 gm. Roentgenograms were made of the femur, tibia, and fibula at the knee joint. Only animals of similar skeletal development were used. It was thought that mitotic activity in these animals would represent mainly regeneration or replacement rather than growth.

Biopsy Technique and Specimen Preparation.—With animals under pentobarbital (nembutal ®) anesthesia, biopsy specimens were taken from the anterior and superior portion of the buccal mucosa. In order to assure uniformity, the hairy cutaneous part of the inner surface of the cheek was taken as a landmark. This area extends from the corner of the mouth posteriorly in a tongue-shaped patch and terminates 5 mm. anterior to the posterior teeth. The boundaries of the patch are very sharp. The biopsy specimen was taken midway between the upper border of the cutaneous patch and the upper fornix of the vestibule and immediately behind the ascending border of the patch.

An incision was made with a circular biopsy instrument of 5 mm. diameter and was carried to a depth of 1.5 mm. The specimen was then severed at its base with fine surgical scissors and immediately fixed in Heidenhain's Susa fluid.[6] To avoid curling of the edges, the tissue was first placed with its deep surface upon a piece of blotting paper soaked in the fixative. After fixation, it was washed, embedded in paraffin, sectioned serially at 6 μ, and stained with alum hematoxylin and eosin. The sections were cut at right angles to the surface of the epithelium.

Normal Biopsies

Time of Biopsy	Number of Specimens *
8 a. m.	8
1 p. m.	8
10 p. m.	8
2 a. m.	9

* Thirty-three animals were used, divided into the four groups shown.

Colchicine Specimens.—Twenty-six animals were given a single intraperitoneal injection of 1 mg. of colchicine salicylate dissolved in distilled water per kilogram of body weight. The concentration of colchicine was 0.5%. All solutions were prepared the day they were used. Specimens for biopsy were taken either three or six hours after the administration of colchicine.

Over 14,000 serial sections were obtained from 41 biopsy specimens taken in eight groups as follows:

Three-Hour Specimens

Time	Number
11 a. m.	5
5 p. m.	5
11 p. m.	5
5 a. m.	6

Six-Hour Specimens

Time	Number
2 p. m.	4
8 p. m.	5
2 a. m.	5
8 a. m.	6

The 11 a. m. and the 2 p. m. groups were unpaired specimens. The other six groups were paired into three sets of three- and six-hour specimens. For these sets each animal was given a single injection of colchicine, and a biopsy specimen was taken on one side three hours later. After six hours a second biopsy specimen was taken in the corresponding area of the contralateral side. Thus each animal served as its own control.

Counting of Cells.—Every fifth section of the serially cut specimen was studied in order to avoid counting the same cell or mitosis twice. In each section five oil immersion fields (magnification 980 diameters) of the epithelium were selected at random. If the selected field included a duct from the buccal salivary glands, it was not used. In order to insure random selection, the microscope was placed slightly out of focus during the change of fields. This obviated the possibility of being attracted to an area because of the presence of a mitosis.

5. According to the manufacturer's analysis, these pellets contain: crude protein, not less than 22.00%; crude fat, not less than 2.50%; crude fiber, not more than 8.50%; nitrogen-free extract, not less than 48.00%.

6. Cowdry, E. V.: Laboratory Technique in Biology and Medicine, Ed. 2, Baltimore, Williams & Wilkins Company, 1948, p. 236.

A count was made of all nuclei that could be clearly seen by focusing through the thickness of the section. Nuclei from the following three zones of the mucosa were included in the count: (1) the layer of basal cells; (2) the adjacent zone of cells which in the investigated area resemble the basal cells in being small, cuboidal in shape, darkly staining, and densely packed (where, as in the epithelial ridges, this zone contains several rows of cells, there is a gradual transition to the type of cells seen in zone 3); (3) a zone of large polyhedral cells which, because of their very lightly staining cytoplasm, have a vesicular appearance. There is a sharp boundary between this zone and the next peripheral one in which the cells are flat and have distinctly pyknotic nuclei.

For each mitosis, the phase, the location of the dividing cell, and the plane of division when possible, were recorded.

Phases of Mitosis.—The phases of mitosis were identified according to the criteria proposed by DeRobertis, Nowinski, and Saez,[7] as follows:

1. Prophase was recognized by an increased size of the cell, an increased basophilia of the nucleus, an increased thickness and visibility of chromatin threads, and a tendency to assume a spherical form. Any mitotic figure in which the nuclear membrane had not begun to disappear was assumed to be in prophase.

2. Metaphase was the term applied to mitotic figures in which the nuclear membrane had begun to disappear but in which divergence of the chromosomes from the equatorial plane had not begun.

3. Anaphase was recognized in a mitotic figure when the chromosomes had begun to diverge from the equatorial plane but the division of the cell body had not started.

4. A mitotic figure was considered to be in telophase from the stage in which division of the cell body could be observed to the stage in which the two daughter cells could barely be distinguished from the surrounding interphasic cells.

Location of Mitoses.—A mitosis was described as belonging to the basal layer of the epithelium if the cell touched the basement membrane and to the stratum spinosum if it did not.

Direction of Division.—The direction of division was classified as being either perpendicular or parallel to the basal layer of the epithelium. Divisions taking place in other planes were classified as perpendicular or parallel depending upon which plane they more nearly approximated. The direction of division could not be determined for cells in prophase. In the case of metaphase it was assumed that the cleavage would take place at right angles to the long axis of the spindle.[8] For cells in anaphase or in telophase, the direction of division could be directly observed.

OBSERVATIONS

A. Mitotic Index.—Normal Biopsies (Table 1A): A total of 596,278 cells showed 3,008 mitotic figures, or a mean mitotic index of 5.1 ± 0.29 mitoses per 1,000 cells for the entire sample of 33 biopsies.

The coefficient of variation was 32.6%.

Three-Hour Colchicine Biopsies (Table 1B): A total of 281,053 cells showed 4,790 mitotic figures, or a mean mitotic index of 17.3 ± 1.38 for the entire sample.

The coefficient of variation for the three-hour colchicine specimens was 35.8%.

The mitotic index in one of the biopsies was 2.9. This figure is lower than that of most normal specimens.

Six-Hour Colchicine Biopsies (Table 1C): A total of 259,478 cells contained 6,607 mitotic figures, or a mean mitotic index of 25.7 ± 2.82, for the entire sample.

The coefficient of variation for the six-hour colchicine sample was 49.0%.

B. Phases of Mitosis.—1. Histologic Effects of Colchicine on Different Phases: After the administration of colchicine, the chromatin material seemed to be more

7. DeRobertis, E. D.; Nowinski, W. W., and Saez, F. A.: General Cytology, Philadelphia, W. B. Saunders Company, 1949, pp. 176-189.

8. Chambers, R.: Structural and Kinetic Aspects of Cell Division, J. Cell & Comp. Physiol. **12**:149-165, 1938.

basophilic in all phases of mitosis. Prophases, anaphases, and telophases appeared essentially normal. The greatest deviation from the normal shape and chromosomal configuration pattern was seen in the metaphase. The chromosomes in this phase usually appeared to be clumped into a nearly spherical mass. Yet, there were many metaphases which could not be distinguished from those seen in normal specimens. Dispersion of chromosomes, in which the chromosomes appeared rather uniformly scattered throughout the cell in early metaphase, was rarely encountered.

2. Frequency of Phases in Normal and Colchicinated Material: (a) Incidence of Phases Among the Cells in Mitosis: All phases of mitosis were seen in all three biopsy groups, and they occurred in the same descending order of frequency: metaphase, prophase, anaphase and telophase. Table 2 shows the percentages of

TABLE 1.—*Composite Data of the Number of Cells, Fields, and Mitotic Index of all Biopsies*

Time	No. Hr.	No. Bio.	No. Cells	No. Fields	Av. No. Cells Per Field	No. Mit.	Av. No. Mit. Per Field	% MI	s	s%	C
A, Normal Biopsies											
8 a. m.	..	8	145,559	1,445	100.7	795	0.55	5.5	0.78	0.26	14.2%
1 p. m.	..	8	134,278	1,358	98.9	970	0.71	7.2	1.59	0.56	22.8%
10 p. m.	..	8	151,571	1,489	101.8	571	0.38	3.8	0.50	0.18	13.1%
2 a. m.	..	9	104,875	1,085	97.2	672	0.40	4.1	1.00	0.30	26.0%
											±19.2
Totals		33	536,278	5,377	99.8	3,008	0.50	5.1	1.66	0.29	32.6
B, Three-Hour Colchicine Biopsies											
11 a. m.	3	5	68,302	750	91.1	1,209	1.61	17.7	4.26	1.91	24.1%
5 p. m.	3	5	70,460	769	91.6	821	1.07	11.6	5.43	2.43	46.8%
11 p. m.	3	5	61,222	765	80.0	1,210	1.58	19.8	8.57	3.92	43.4%
5 a. m.	3	6	81,050	900	90.1	1,560	1.73	19.1	2.71	1.10	14.2%
											±32.1
Totals		21	281,053	3,184	88.3	4,790	1.5	17.3	6.2	1.38	35.8
C, Six-Hour Colchicine Biopsies											
8 a. m.	6	6	70,000	895	88.3	2,721	3.04	34.4	14.4	5.77	41.1%
2 p. m.	6	4	49,986	580	86.2	642	1.11	12.8	3.91	1.95	30.5%
8 p. m.	6	5	62,755	780	82.6	1,545	2.08	24.6	1.91	0.85	7.8%
2 a. m.	6	5	67,732	785	86.3	1,690	2.18	28.3	16.4	7.34	62.4%
											±35.5
Totals		20	250,473	3,039	85.9	6,607	2.19	25.7	12.6	2.82	49.0

phases for each biopsy. In the colchicine specimens, metaphases constitute a higher fraction of the total number of mitoses; prophases and anaphases and telophases correspondingly lower fractions, particularly in the specimens with high mitotic indices.

(b) Incidence of Phases Among the Total Cell Population: For each of the three groups of biopsies, the percentage of cells in each phase of mitosis and in interphase was computed (Table 3). During three hours of colchicine action the percentage of cells in interphase was decreased from 99.5 to 98.3% and during six hours from 99.5 to 97.4%. The corresponding percentage increases in the frequency of cells in mitosis were found to be due almost wholly to increases in metaphases, which constituted 0.23% of all cells in the normal material, 1.47% of the cells in the three-hour colchicine specimens, and 2.30% of the cells in the six-hour colchicine specimens. There was practically no change in the percentage figures of anaphases,

TABLE 2.—*Percentage Distribution of the Phases of Mitosis in Individual Biopsies*

No. of Biopsy	Time of Biopsy	% Prophases	% Metaphases	% Anaphases + % Telophases	Mitotic Index
	A. Percentage Distribution of Phases in Normal Biopsies				
33	10 p. m.	25.0	55.0	20.0	2.9
34		56.3	21.8	21.9	3.2
32		17.7	61.3	21.0	3.3
31		28.6	55.9	15.5	3.8
36		31.8	44.0	24.2	3.9
37		23.2	57.3	19.5	4.0
35		25.4	53.7	20.9	4.0
30		25.3	56.5	19.2	4.4
42	2 a. m.	56.6	24.5	18.9	2.6
44		41.5	49.1	9.4	3.3
43		65.5	20.7	13.8	3.7
45		56.8	25.6	17.6	3.8
41		43.8	38.5	17.7	3.8
40		45.6	27.7	26.7	3.9
46		33.3	50.0	16.7	4.6
48		43.9	39.0	17.1	4.9
47		32.1	57.5	10.4	6.4
66	8 a. m.	40.5	38.0	21.5	4.5
64		39.7	32.1	28.2	4.9
62		39.3	41.9	18.8	5.1
60		27.3	53.5	19.2	5.3
67		24.8	59.3	15.9	5.6
61		39.8	41.8	18.4	5.8
63		38.3	40.2	21.5	6.0
65		36.5	54.8	8.7	6.5
53	1 p. m.	27.9	54.1	18.0	3.6
56		28.0	41.7	29.3	6.6
57		30.4	51.3	18.4	7.0
54		31.0	37.3	31.7	7.5
50		29.2	54.8	16.0	8.1
55		21.9	57.5	20.6	8.1
52		46.7	27.1	26.2	8.3
51		22.7	54.6	22.7	8.4
	B. Percentage Distribution of Phases in 3-Hour Biopsies				
6c	5 p. m.	24.5	69.4	6.1	2.9
10c		23.5	62.3	14.2	11.6
7c		9.1	86.3	4.6	14.5
8c		8.0	82.0	9.0	15.7
9c		6.4	88.7	4.9	15.9
21	11 a. m.	6.6	86.8	6.6	12.0
26		11.8	79.2	9.0	15.0
24		4.3	90.1	5.6	16.9
23		1.2	96.9	1.9	21.4
22		8.1	82.3	5.6	23.1
19	5 a. m.	1.4	93.1	5.5	15.9
16		9.5	76.7	13.8	16.3
17		1.5	95.9	2.6	19.5
18		4.3	91.0	4.7	19.7
25		4.4	85.2	10.4	20.6
20		2.6	96.8	1.6	28.1
13ca	11 p. m.	19.4	72.4	8.2	11.2
15ca		10.0	84.5	5.5	14.8
14ca		9.5	82.1	8.4	17.0
12ca		11.8	82.7	5.5	22.4
11ca		5.1	85.0	9.9	33.7
	C. Percentage Distribution of Phases in 6-Hour Biopsies				
1c	2 p. m.	14.7	77.9	7.4	8.9
4c		9.7	75.0	15.3	11.1
2c		8.1	88.6	3.3	13.8
5c		11.1	85.2	3.7	16.9
7ca	8 p. m.	18.2	71.9	9.9	15.9
6ca		14.7	78.7	6.6	21.0
9ca		4.3	90.4	5.3	24.9
8ca		4.0	87.3	8.7	28.1
10ca		1.4	94.3	4.3	33.4
13ca	2 a. m.	13.0	76.7	10.3	10.5
12ca		13.0	80.0	7.0	16.4
14ca		6.3	82.6	11.1	17.0
15ca		1.6	90.7	7.7	40.1
11ca		0.8	97.5	1.7	47.6
25a	8 a. m.	10.8	71.9	17.3	13.6
20a		1.7	96.6	2.7	21.9
18a		3.4	93.2	3.4	33.5
16a		0.3	99.2	0.5	42.7
19a		1.2	95.5	3.3	46.5
17a		0.5	99.2	0.3	49.7

which constituted 0.07% in the normal, 0.08% in the three-hour samples, and 0.09% in the six-hour samples. Similarly, the percentages of cells in telophase were practically constant, amounting to 0.03% in the normal, 0.03% in the three-hour, and 0.04% in the six-hour colchicine material. The percentage of prophases, on the other hand, was found significantly and evenly lowered in the colchicinated material: Prophases constituted 0.17% of all cells in the normal specimens, 0.12% in the three-hour, and 0.12% in the six-hour colchicine specimens.

C. Location of Mitosis.—Of the 3,008 mitoses observed in the normal biopsies, 59.8% were in the basal layer and 40.2% in the inner layers of the stratum spinosum (Table 4). This difference was statistically significant.

There was a definite decrease in the percentage of mitoses seen in the basal layer in the three-hour colchicine biopsies, and further decrease was noted in the six-hour colchicine biopsies. After three hours of colchicine administration, the mitoses in

TABLE 3.—*Number and Percentage of Cells in Various Stages of Mitosis*

Type of Biopsy	Cells, No.	Interphase		Prophase		Metaphase		Anaphase		Telophase	
		No.	%	No.	%	No.	%	No.	%	No.	%
Normal	566,278	560,270	98.49	1,040	0.17	1,380	0.23	438	0.07	150	0.03
3-hr. colchicine	281,053	276,263	98.29	343	0.12	4,128	1.47	227	0.08	92	0.03
6-hr. colchicine	259,478	252,871	97.45	305	0.12	5,973	2.30	231	0.09	98	0.04

TABLE 4.—*Number and Percentage of Mitoses in Basal Layer and in Stratum Spinosum of Stratum Germinativum*

Type of Biopsy	Mitoses, No.	Basal Layer		Stratum Spinosum	
		No.	%	No.	%
Normal	3,008	1,798	59.77	1,210	40.23
3-hr. colchicine	4,790	2,472	51.60	2,318	48.40
6-hr. colchicine	6,607	3,144	47.58	3,293	52.42

the basal layer represented only 51.6%, and after six hours only 47.6% of the total number of mitoses observed (Table 4).

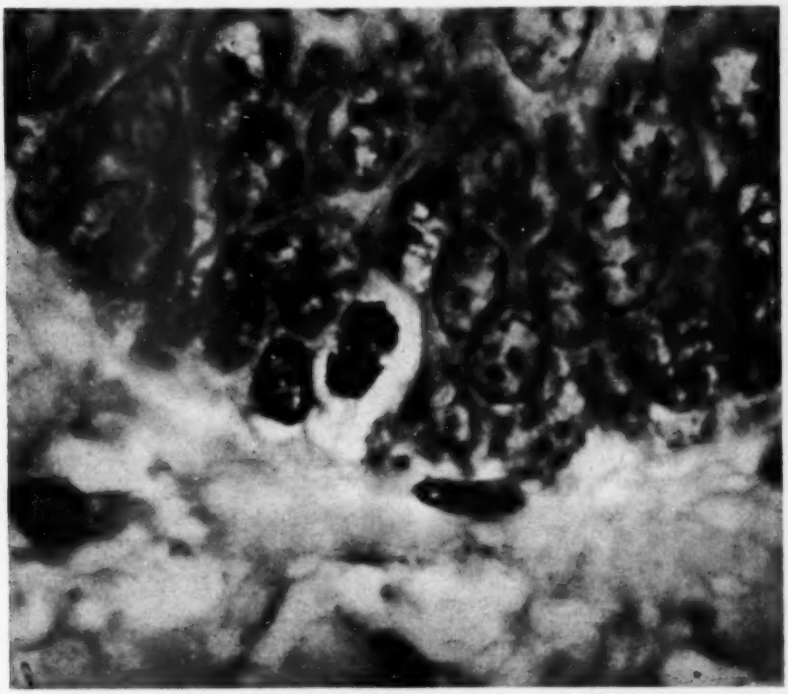
Although the stratum spinosum cannot be divided into sharply differentiated zones, it was noted that there was virtually no mitotic activity among the polyhedral vesicular cells in the outer zone of the stratum spinosum.

D. Direction of Cell Division in the Basal Layer.—An attempt was made to determine the plane of division in the basal layer for metaphases, anaphases, and telophases. However, many instances arose in which the direction of division of metaphases was in doubt. For this reason, only anaphases and telophases were recorded.

The three groups of biopsies showed a total of 833 anaphases and telophases located in the basal layer. Of these, more than half (54.9%) were dividing in a plane that was approximately at right angles to the basement membrane (Fig.). The remainder (45.1%) were dividing in a plane approximately parallel to the basement membrane (Table 5).

E. *Frequency Distribution of Mitoses per Oil Immersion Field.*—The average number of mitoses per oil immersion field was 0.5 for normal biopsies, 1.5 for 3-hour colchicine biopsies, and 2.2 for six-hour colchicine biopsies (Table 1).

The largest number of mitoses seen in a single field was 6 in the normal, 8 in the three-hour colchicine, and 12 in the 6-hour colchicine material. Four and more mitoses per field were seen in 0.5% of the normal fields, 8.6% of the 3-hour, and 20.4% of the six-hour colchicine fields examined. One, two, or three mitoses per field were seen in 36.0% of the normal, 64.0% of the three-hour, and 60.0% of the six-hour colchicine specimens. Two or more mitoses were seen in 10.9% of the normal fields. No mitoses were seen in 63.5% of the normal, 27.1% of the three-hour, and 19.6% of the six-hour colchicine fields.



Oral epithelium of a normal rabbit, showing anaphase figure with equatorial plate parallel to the basement membrane; × 1,600.

TABLE 5.—*Number and Percentage of Anaphases and Telophases in Basal Layer Dividing Parallel and Perpendicular (All Specimens)*

	Parallel		Perpendicular	
Anaphases + Telophases	Number	Percentage	Number	Percentage
833	376	45.13	457	54.87

COMPUTATIONS AND INTERPRETATIONS

A. Mitotic Index.—The number of cells in mitosis per thousand cells examined was 5.1 ± 0.29 (Table 1A).

This value was established from specimens taken at representative times of the 24-hour cycle. The diurnal variation in mitotic activity ranged from a minimum of 3.8 ± 0.18 mitoses per 1,000 at 10 p. m. to a maximum of 7.2 ± 0.56 at 1 p. m. This variation is chiefly responsible for the very high coefficients of variation found in both the normal and the colchicinated specimens.

B. Effect of Colchicine on the Accumulation of Mitoses.—The difference between the mitotic indices of colchicinated and normal specimens measures the rate at which mitoses accumulate during the period of colchicination. During the 24-hour daily cycle, subdivided into four periods of six hours each, an average mitotic index of 25.5 was seen in colchicinated material. Biopsies of normal animals covering representative hours of the daily cycle showed an average mitotic index of 5.1. The average rate of accumulation, therefore, was 20.4 cells per 1,000 during six hours of colchicine action, or 3.4 cells in a 1,000 entering mitosis every hour. The same rate of accumulation can be computed from the reduced percentage of cells in interphase. Table 3 showed 99.49% cells in interphase in the normal material and 97.45% in the six-hour colchicine material. The difference between these two percentages is 2.04% in six hours, or 0.34% per hour.

C. Effect of Colchicine During the First and Second Three Hours of Action.—In order to appraise whether the rate of accumulation continued unaltered for six hours, a paired comparison was made for those of the three- and six-hour specimens in which each animal was used as its own control. For the three paired six-hour groups of specimens, the average mitotic index was a little higher than for the complete six-hour sample (28.9 instead of 25.7), since the unpaired six-hour group of specimens showed a very low rate of accumulation. For the three paired three-hour groups of specimens, the average mitotic index was almost exactly the same as for the complete three-hour sample (17.2 instead of 17.3). The average difference for the paired three- and six-hour specimens (28.93 — 17.15) is 11.8. Thus, an average of 11.8 mitoses per 1,000 cells accumulated during the second three hours of colchicine action. The difference between the three-hour specimens and the normal material was 17.3 — 5.1, or 12.2 mitoses per 1,000 cells.

The first and second three hours of colchicine action thus showed practically identical rates of accumulation of mitoses.

D. Effect of Colchicine on the Incidence of Prophase.—In the two groups of colchicinated biopsies, only 0.12% of all cells were found to be in prophase, as compared with 0.17% of the cell total in normal material (Table 3). Colchicine, therefore, reduced the incidence of prophase to 70.6% (12/17) of its value in the normal animals.

E. Rate of Regeneration of the Oral Mucosa.—The rate of accumulation of mitoses in colchicinated animals measures the rate of entrance of cells into mitosis in the normal animal, provided that two conditions are met: (1) that all mitoses initiated since administration of the drug are arrested for the period of observation or that the degree of escape from arrest can be estimated, and (2) that the rate of entrance into mitosis is not altered by colchicinization, or, again, that the degree of alteration can be estimated.

Our observations provide no means of estimating whether the first condition is satisfied, and the ensuing uncertainty will be discussed below. As regards the second condition, the data on incidence of prophase can be used to estimate the degree

to which colchicine alters the rate of entrance into mitosis. Since there is no reason to assume that colchicine decreases the duration of prophase, a lowered percentage of cells in the earliest stage of division indicates that the rate at which cells enter division is depressed. This depression amounted to 29.4% (5/17). The rate of accumulation of mitoses was seen to be 0.34% per hour. Correction for the depression caused by colchicine raises this value to $0.34\% \times 17/12 = 0.48\%$.

Thus, the rate at which the cells of the oral epithelium enter mitosis is 0.48% per hour. The period required for replacement of the entire epithelium is 100/0.48, or 208 hours. This period is identical with the average life span, or the intermitotic time, of the cells.

F. Duration of Mitosis.—The establishment of an average mitotic index and an average duration of interphase permits the computation of the average duration of mitosis. The ratio between the number of cells in mitosis and the number of cells in interphase must be the same as the ratio between the duration of mitosis and the duration of interphase, or $\frac{\text{number of cells in mitosis}}{\text{number of cells in interphase}} = \frac{\text{duration of mitosis}}{\text{duration of interphase}}$

For our material, the figures are $0.51/99.49 = x/208$. The value of x in this equation is 64.1 minutes. The average duration of mitosis, therefore, is 64 minutes.

G. Effect of Colchicine on Cell Division in Basal and Spinous Layer.—Our data show 59.8% of all mitoses located in the basal layer in the normal material, 51.6% in this layer after three hours of colchicine, and 47.6% after six hours of colchicine (Table 4). A similar decrease in the percentage of mitoses in the basal layer can be computed from Cowdry's figures for the plantar epidermis in mice [9]: 28.0% of all mitoses were located in the basal layer in his normal material, and 21.3% were located there after eight hours of colchicine action.

Colchicine thus depresses entrance of the basal cells into mitosis more markedly than that of the spinous cells, indicating a difference in the response of the two types of cells. This differential effect continues in the second three-hour period of the action of colchicine, and therefore can hardly be attributed to the circumstance that the drug reaches the basal cells sooner.

H. Frequency Distribution of Mitoses.—In the normal material, 0.5% of the fields examined had four or more mitoses; 10.9% had two or more mitoses; 63.5% of the fields, on the other hand, showed none. This indicates a certain tendency for mitoses to cluster, a fact which has also been observed in epidermal epithelium.[10]

The distribution of mitoses in colchicinated material in comparison with normal material furnishes information on whether mitotic activity remains located in the same area or migrates to other regions over a period of six hours.

Only 0.5% of the normal fields contained four or more mitoses, compared with 8.6% and 20.4% of the three- and six-hour colchicine fields. Presumably the

9. Cowdry, E. V., and Thompson, H. C., Jr.: Localization of Maximum Cell Division in Epidermis, Anat. Rec. **88**:403-409, 1944.

10. (*a*) Flemming, W.: Zur Kenntniss der Regeneration der Epidermis beim Säugethier, **Arch. mikr. Anat. 22**:5-148, 1884; (*b*) Über die Regeneration verschiedener Epithelien durch mitotische Zellteilung, ibid. **24**:372, 1885. (*c*) Thuringer,[2a] (*d*) Broders, A. C., and Dublin, W. B.: Rhythmicity of Mitosis in Epidermis of Human Beings, Proc. Staff Meet., Mayo Clin. **14**:423-425, 1939.

increase in the percentage of fields with many mitoses comes about by continued mitotic activity in fields which already had some mitoses in the normal material. There is, on the other hand, an increase from 36.0 to 64% and to 60% in the frequency of fields with one to three mitoses, and a decline from 63.5% to 27.1% and 19.6% in the frequency of fields with no mitoses. Both the normal fields with one to three and those with no mitoses must have contributed to the increase in the number of fields with one to three mitoses in colchicinated material. This means that there was arrest of mitotic activity in the former and entrance into mitosis in the latter fields.

It appears that at a given moment mitotic activity is somewhat unevenly distributed. However, shifts in the location of the regions of higher activity occur within a matter of hours.

COMMENT

The main purpose of this study was to determine the rate at which the oral epithelium regenerates in the rabbit. This was done by means of colchicine. It was first established that the action of this drug on mitosis is uniform over a six-hour period. Next, a 24-hour period was subdivided into four intervals of six hours each and the mitotic index for each of these intervals established in colchicinated animals. Further, the mitotic index of normal animals was established for representative times of the 24-hour cycle. The difference between the average index under colchicine and the average index in the normal animal is the average rate at which mitoses accumulate during six hours. From the six-hour figure, the rate at which mitoses accumulate in one hour was computed. On the assumption that all mitoses are arrested by colchicine, the rate of accumulation per hour gives the rate at which cells enter mitosis in colchicinated animals. Since colchicine has a depressing effect on entrance into mitosis, the rate found in colchicinated animals must be corrected in proportion to this depression in order to establish the rate at which cells enter mitosis in the normal animal.

From the hourly rate of entrance into mitosis one can, of course, readily calculate how long it would take to double the number of cells present. In the fully grown animal, the number of cells in the mucosal epithelium of a given area can be taken to remain constant. The time for doubling the number of cells then becomes the time it takes to replace the number of cells present, and mitotic activity serves to compensate for the loss of cells in the superficial layers. The daughter cells of a cell dividing in the reproducing layer may either become members of the surface layers in which no mitoses occur (postmitotic cell in Cowdry's sense)[11] or remain in the germinative layer and there undergo further mitosis (vegetative intermitotics and differentiating intermitotics of Cowdry). If cells of the reproducing layer participate evenly in the reproductive activity, that is, if they all divide and the interval between successive divisions is the same for all, then the time for complete replacement is identical with the life span of the individual epithelial cell. Depending on the fate of the daughter cells, the life span measures either the time between mitoses (intermitotic time) or the time from the last mitosis to departure of the cell from the germinative layer. If participation in mitotic activity is not uniform, the time for

11. Cowdry, E. V.: Ageing of Individual Cells, in Problems of Ageing: Biological and Medical Aspects, edited by E. V. Cowdry, Baltimore, Williams & Wilkins Company, 1942.

100% replacement merely measures the average life span or average intermitotic time. The data on distribution of mitoses over the microscopic fields show that participation in the mitotic process is fairly uniform among the cells of a given layer.

Below, the use of colchicine for determining the rate of regeneration will be discussed. The results obtained with this method will be compared with results obtained by a different experimental approach. Only a very small number of such studies have been carried out, however. It was therefore thought worth while to precede the discussion of rates of regeneration with a comparison of mitotic indices, on which considerably more information is available.

A. Mitotic Index.—The oral mucosa has not previously been the object of a quantitative study of mitotic activity; hence a comparison of the findings in the rabbit with those in other animals cannot be made. For this reason, data on mitotic

TABLE 6.—*Mitotic Indices of Epithelia, Mitoses per 1,000 Cells*

Item No.	Author	Year	Body Region	Species	Age	Time of Biopsy	Mitotic Index	Comment
			Epidermis					
1	Thuringer	1928	Prepuce	Man	Infant	Not stated	5.61	.....
2	Broder	1939	Prepuce	Man	Infant	14 samples 9 a. m.-11 p. m.	7.14	Computed
3	Cooper	1938	Prepuce	Man	Infant	Over 24 hr.	3.81	Computed
4	Carleton	1933	Abdomen	Mouse	1-7 days	Over 24 hr.	9.8	Computed
5	Cowdry	1944	Foot pad	Mouse	10 days	4 a. m.	26.8	Computed
6	Thuringer	1928	Prepuce	Man	3 years	Not stated	0.74	.....
7	Thuringer	1928	Scalp	Man	Adult	Not stated	0.41	.....
8	Thuringer	1928	Ear	Man	Adult	Not stated	0.0037	.....
9	Thuringer	1928	Leg	Man	Adult	Not stated	0.0026	.....
10	Thuringer	1928	Foot pad	Cat	Adult	Early a. m.	2.37	.....
11	Knowlton	1950	Ear	Mouse	42-56 days	Not stated	0.75	.....
12	Knowlton	1949	Ear	Mouse	42-56 days	Not stated	1.69	.....
13	Cooper	1940	Ear	Mouse	50 days	Over 24 hr.	1.45	Computed
			Mucosa					
14	Present study	....	Oral cavity	Rabbit	100 days	Over 24 hr.	5.1	.....
15	Knowlton	1950	Crypts, jejunum	Mouse	42-56 days	Not stated	9.3	.....
16	Widner	1951	Crypts, jejunum	Rat	Fully grown	Not stated	13.7	.....
17	Leblond	1948	Crypts & villi, duodenum	Rat	Adult	11:00 a. m.	33.0	.....

indices of other epithelia were assembled (Table 6), in the expectation that some order would emerge in which the present data could find their place. In Table 6 are included those quantitative studies of mitotic activity for which mitotic indices were given. In addition, some indices were calculated from data published on diurnal variation of mitotic activity.

Mitotic indices of several cells per thousand occur regularly in the epidermis of very young organisms (items 1, 2, 3, 4, and 5 in Table 6). Among older animals, they occur only in the oral mucosa (item 14) and in the intestinal epithelium (items 15, 16, and 17). In all specimens beyond early infancy, epidermal epithelium seems to be of a lower order of mitotic activity (items 6, 7, 8, 9, 11, 12, and 13). Only regions under very high functional demands, such as the plantar epidermis (item 10), approximate the level seen in the oral mucosa. It is not surprising to find the highest epidermal index in functionally hyperplastic skin in extreme youth (item 5). Comparison of the data on the epithelium of the ear and that of crypts and villi of the intestine in mice and rats (compare items 11, 12, and 13 with items

15, 16, and 17) leaves little doubt that a much higher percentage of the cell population is in mitosis in the intestinal mucosa than in the epidermal epithelium. The oral mucosa appears to occupy an intermediary position between these two types.

There are some discrepancies between the findings of different workers examining the same object. While one source of variation, the time interval between removal and fixation of the specimens, was recognized, and the interval made as brief as possible by almost all authors, the diurnal variation of mitotic activity has not always been taken into account. In the present material, the mitotic index was twice as high at the peak of the daily cycle as at the minimum. In the material of other authors, the diurnal variation was even more marked.[12] This source of variability may easily explain the discrepancies between items 1, 2, and 3 of Table 6, also between items 11 and 13. It may have a share in explaining the marked discrepancy between items 15 and 16 on the one hand, and item 17 on the other. As will be seen below, differences in the criteria used to define mitosis are likely to be of greater importance in the last instance. This factor, as well as variations due to the estrous cycle in female animals,[13] may contribute to other discrepancies in the findings.

B. The Use of Colchicine for Determining Rate of Entrance into Mitosis.—The use of colchicine to determine the rate of entrance into mitosis was proposed by Allen [14] in the study of hormonal influences on mitotic activity after it was discovered that colchicine arrests mitotic division in metaphase.[15] It was extensively used also by Brues [16] in studying repair processes in the liver. Since these earlier appli-

12. Carleton, A.: Rhythmical Periodicity in Animal Cells, J. Anat. **68**:251-263, 1933. Cooper, Z. K., and Franklin, H. C.: Mitotic Rhythm in Epidermis of Mouse, Anat. Rec. **78**:1-8, 1940. Cooper, Z. K., and Schiff, E. A.: Mitotic Rhythm in Human Epidermis, Proc. Soc. Exper. Biol. & Med. **39**:323-324, 1938. Blumenfeld, C. M.: Periodic Mitotic Activity in Epidermis of Albino Rat, Science **90**:446-447, 1939; Relationship of Function, Light and Temperature to Growth by Mitosis, Arch. Path. **38**:321-325, 1944. Bullough, W. S.: Mitotic Activity in the Adult Male Mouse, Mus Musculus L.: The Diurnal Cycles and Their Relation to Waking and Sleeping, Proc. Roy. Soc. s.B **135**:212-233, 1947; The Effects of Experimentally Induced Rest and Exercise on the Epidermal Mitotic Activity of the Adult Male Mouse, Mus Musculus L., ibid. **135**:233-242, 1948; Age and Mitotic Activity in the Male Mouse, Mus Musculus L., J. Exper. Biol. **26**:261, 1949.

13. Bullough, W. S.: Epidermal Mitotic Activity in the Adult Female Mouse, J. Endocrinol. **6**:340-349, 1950; The Mitogenic Actions of Starch and Oestrone on the Epidermis of the Adult Mouse, ibid. **6**:350-361, 1950.

14. Allen, E.; Smith, G. M., and Gardner, W. U.: Accentuation of the Growth Effect of Theelin on Genital Tissues of the Ovariectomized Mouse by Arrest ot Mitosis with Colchicine, Am. J. Anat. **61**:321-342, 1937.

15. (*a*) Dustin, A. P.: Contribution à l'étude de l'action des poisons caryoclasiques sur les tumeurs animals: Deuxième mémoire; Action de la colchicine sur le sarcome greffé, type Crocker, de la souris, Bull. Acad. roy. méd. Belgique **14**:487-502, 1934. (*b*) Lits, F. J.: Contribution à l'étude des réactions cellulaires provoquées par la colchicine, Compt. rend. Soc. biol. **115**:1441-1443, 1934.

16. (*a*) Brues, A. M.: The Effect of Colchicine on Regenerating Liver, Physiology **86**:63, 1936. (*b*) Brues, A. M., and Cohen, A.: Effects of Colchicine and Related Substances on Cell Division, Biochem. J. **30**:1363-1368, 1936. (*c*) Brues, A. M., and Jackson, E. B.: Nuclear Abnormalities Resulting from Inhibition of Mitosis by Colchicine and Other Substances, Am. J. Cancer **30**:504-511, 1937. (*d*) Brues, A. M., and Marble, B. B.: Analysis of Mitosis in Liver Restoration, J. Exper. Med. **65**:15-27, 1937.

cations, it has become increasingly clear that colchicine is not a perfect tool for this purpose,[17] for three reasons: (1) Even under optimal dosage it may alter the rate at which cells enter mitosis; (2) some cells may escape the blocking effect and complete their division, and (3) even among littermates there is considerable variation in the response to colchicine.

Bucher's cinematographic and microscopic observations on tissue cultures showed that a dose of colchicine which is so high that it markedly depresses the entrance of cells into mitosis is not high enough to block all dividing cells in metaphase. With lower doses there is even more escape from metaphase arrest, though the rate of entrance into mitosis may be nearly normal. Complete arrest in metaphase is achieved only when the dose given is so high that almost no new cells enter into mitosis. Furthermore, the same dose produces variable effects when given to several specimens of the same tissue culture. Brues' definition of the optimal dose of colchicine [16b] as "the average amount which gives a maximum number of abnormal metaphases, i. e., which is effective in stopping mitosis in metaphase, but which does not prevent cells from entering mitosis," appears over-optimistic in the light of Bucher's observations, which make it clear that no dosage can satisfy more than one of Brues' two conditions.

In the present study, a dose of 1 mg. per kilogram was used. This dose had been selected in a pilot experiment using 27 rabbits, because it seemed to yield the highest average accumulation of metaphases. As 1 mg. per kilogram is the amount most commonly used in other species, it seems that rabbits do not differ from rodents in their response to colchicine. A lower dose was used by Cowdry in mice [9] and yielded only twice the normal mitotic index during eight hours of accumulation. Similarly, Carleton [17a] obtained only a slight accumulation of mitoses when using 0.5 mg. per kilogram in young rats. In our material, the lowest accumulation of mitoses occurred in those specimens in which prophase was least depressed (Table 2 B and C). Thus, the cause for low rates of accumulation was not the depressed rate of entrance into mitosis, but rather incomplete blockage in metaphase. Only in one animal was a low incidence of prophase associated with a low mitotic index. Conversely, the highest mitotic indices in a group of specimens were commonly associated with the lowest incidences of prophase. High rates of accumulation thus were not due to undisturbed entrance into mitosis but, rather, to more nearly complete arrest in metaphase.

These findings make it apparent that our material is subject to all three sources of error inherent in the use of colchicine. The magnitude of the first of these errors can be estimated by comparing the incidence of prophase in normal and colchicinated material. This was done in the computations presented, and a corrected rate of entrance into mitosis was obtained.

The second source of error, the escape from arrest in metaphase, cannot be well estimated. There is little information on the incidence of anaphases and telophases

17. (*a*) Carleton, A.: Effect of Colchicine on Skin of Young Rats, J. Anat. **73**:416-418, 1938. (*b*) Bucher, O.: Zur Kenntnis der Mitose: VI. Der Einfluss von Colchicin und Trypaflavin auf den Wachstumsrhythmus und auf die Zellteilung in Fibrocytenkulturen, Ztschr. Zellforsch. u. mikr. Anat. **29**:283-322, 1939. (*c*) Cowdry.[9] (*d*) Leblond and Stevens.[4a] (*e*) Bullough, W. S.: The Action of Colchicine in Arresting Epidermal Mitoses, J. Exper. Biol. **26**:287-291, 1949.

in colchicinated material or on whether cells in these stages complete mitosis in the colchicinated animal. The present findings show a high positive correlation between the percentages for prophases and those for anaphases plus telophases. But the quantitative relationship between the two percentages is not a constant one and therefore not usable for working out a correction factor. The incidence of abnormal-looking metaphases also fails to provide a means for estimating the degree of arrest. Normal-looking metaphases occur in all but the highest concentrations of colchicine,[18] but many of these histologically normal metaphases may not complete mitosis during the experimental period.[17b]

Short of estimating its magnitude, one can indicate the direction of the error caused by escape from metaphase arrest. Since all mitoses which come to completion during the experimental period drop out from the mitotic count, escape from colchicine arrest leads to lowered mitotic indices and thereby to underestimation of the rate of entrance into mitosis.

An error in the same direction is caused by the variable response to colchicine. Both the severer depression of entrance into mitosis in the hypersensitive animal and the higher escape from metaphase arrest in the hyposensitive animal result in a less than maximal accumulation of mitoses.

The actual rate of physiologic regeneration, therefore, is likely to be faster than its estimate by the use of colchicine. Applied to the present findings, this means that the estimated time for complete regeneration of the oral epithelium of the rabbit is less than 208 hours.

C. Other Methods for Determining Rates of Regeneration.—In the above presentation of findings, the following relationship was used to calculate the duration of mitosis: $\frac{\text{number of cells in mitosis}}{\text{number of cells in interphase}} = \frac{\text{duration of mitosis}}{\text{duration of interphase}}$ The same equation could, of course, be used to calculate the duration of interphase if the duration of mitosis were known. If it were possible to observe the duration of mitosis in vivo, this would be a much simpler way of determining rates of regeneration than the laborious method of establishing rates of accumulation of mitoses under colchicine.

Because direct measurement of the duration of mitosis in the animal is not feasible, some authors have instead used observations made on tissue cultures. Hoffmann,[19] e. g., has computed rates of regeneration for the basal and spinous cells of the human epidermis based on the mitotic counts made by Thuringer in 1928 and on various estimates of the duration of mitosis made on tissue cultures. Hoffmann himself has pointed out that his computations are unreliable to the degree to which these estimates of mitotic duration are unreliable, or rather, inapplicable.

Knowlton and associates [20] have recently developed a method for estimating duration of mitosis by means of x-rays. When moderate doses are given to an animal, the stage of early prophase is so protracted that, according to the authors,

18. Brues and Cohen,[16b] Bucher,[17b] Leblond and Stevens.[8a]

19. Hoffmann, J. G.: Quantitative Analysis of the Growth of the Epidermis, Arch. Path. **47**:37-43, 1949.

20. Knowlton, N. P., Jr.; Hempelmann, L. H., and Hoffman, J. G.: The Effect of X-Rays on the Mitotic Activity of Mouse Epidermis, Science **107**:625-626, 1948. Footnote 4*c* and *d*.

only negligible numbers of cells progress beyond this phase during the observational period. Mitotic indices exclusive of early prophase are determined in successive brief intervals after irradiation, and they show a regular decrease with increasing time intervals. By extrapolating the curve of mitotic indices so obtained to the time when the mitotic index would have been zero, the authors obtained an estimate of the duration of mitosis, which they used for computing the intermitotic time. Results obtained by this method will in the next section be compared with results obtained with the use of colchicine.

D. Rate of Regeneration of the Oral Mucosa.—The few estimates in the literature of rates of regeneration of epithelia under physiological conditions are compared in Table 7 (in terms of hours for 100% replacement or hours of intermitotic time). The oral mucosa is seen to occupy a position between epidermis and intestinal mucosa, just as was found with respect to its mitotic index.

TABLE 7.—*Rates of Regeneration of Epithelia*

Author	Year	Species	Region	Mitotic Time, Min.	Mitotic Index (Mitoses per 1,000 Cells)	Intermitotic Time, Hr.
Leblond	1948	Adult rat	Duodenum	68 (colchicine)	23.0	36.8
Widner	1951	Fully grown rat	Jejunum	27.5 (x-ray)	13.7	33.5
Knowlton	1950	Mouse, 6-8 weeks	Jejunum	24 (x-ray)	9.3	43
Present study	....	Rabbit	Oral mucosa	64 (colchicine)	5.1	208
Knowlton	1950	Mouse, 6-8 weeks	Epidermis of ear	30.2 (x-ray)	0.75	670
Hoffman	1940	Adult human	Scalp	30 (estimated)	0.41	740 for spinous cells 3,100 for basal cells

The rate of regeneration of intestinal mucosa in rats established with the use of colchicine by Leblond [48] agrees well with the rate estimated with the use of x-rays by Widner.[61] However, a comparison of the mitotic indices and duration of mitosis established in the two studies shows wide discrepancies. Evidently Leblond includes earlier and later stages of mitosis than Widner, thereby obtaining higher indices and longer duration of mitosis. As this difference in the criteria used to define mitosis affects index and duration in the same manner, it cancels out in calculating the rate of regeneration. But agreement on rates of regeneration cannot be expected when the two experimentally determined variables are not established by the same worker.

E. Role of Basal and Spinous Cells in the Physiologic Regeneration of Epithelia.—The earlier literature tended to consider only the basal and perhaps the innermost spinous layer as the germinative strata of epithelium. Thuringer, in reporting his series of findings in 1924, 1928, and 1939, gives an extensive review of the literature preceding his studies. His own findings led him to conclude that in the adult, under physiologic conditions and in regions in which functional demands are moderate, the basal cells are concerned with maintenance of merely the basal layer, whereas the mitotic activity of spinous cells preserves the status quo of the rest of the epithelium against the continuous losses at the surface.

Thuringer's conclusions are based on mitotic counts in the basal and spinous layers and on observations of the plane of cell division in the basal layer. Table 8 summarizes his findings on the distribution of mitoses within the epithelium, as well as the few studies made by other authors since then.[21] Regarding the relative frequency of mitosis in the basal layer, considerable variation for epidermal epithelia of different ages and body regions has been observed (8 to 44.5% of the total mitotic count). The proportion of mitoses in the basal layer of the oral mucosa (60%), however, seems to fall outside the range of variation seen in the epidermis.

As regards the direction of cell division in the basal layer, which Thuringer [2b] found invariably so oriented as to make the daughter cells members of the basal layer, the oral mucosa again behaves in a different manner: Forty-five per cent of the mitoses in the basal layer, or 27% of the mitotic count of the entire epithelium, are so oriented as to place one of the daughter cells directly into the stratum spinosum (Fig). In view of the very high relative frequency of cell division in the basal layer it seems evident that, in addition, cells from the basal layer dividing in other directions must pass into the stratum spinosum in a less direct manner.

TABLE 8.—*Distribution of Mitoses Within Epithelium*

					Distribution, %				
Author	Year	Species	Age	Body Region	Basal	Inner	Middle	Outer	Total Spinous
Thuringer	1928	Man	Infant	Prepuce	8.33	76.1	15.35	0.22	91.67
Thuringer	1934	Man	Adult	Scalp	12	50	40	12.00	88.0
Cowdry	1944	Mouse	10 days	Foot pad	27.5	46.1	19.8	6.5	72.5
Thuringer	1930	Cat	Adult (?)	Foot pad	44.5	54.5	1.0	0	55.5
Brauer	1933	Cat, sheep	Adult	Bladder	20.3	30.1	36.6	13.0	79.7
Present study	....	Rabbit	Adult	Oral cavity	60	....	.....	....	40

Thuringer's conclusion that the sole function of mitosis among basal cells is the maintenance of the basal layer can therefore not be extended to the oral mucosa of the rabbit.

The attempt to specify location of cell division within the spinous layer had to be abandoned in the present study, because of the irregular configuration of the epithelial ridges in the area studied. Many mitotic figures which in one section appeared to be in the middle or outer third of the stratum spinosum were found located adjacent to the basal layer when the next serial section was examined. It can, however, be stated that mitotic activity was confined to the darkly staining small cells of the inner zone of the stratum spinosum which closely resemble the cells of the basal layer.

SUMMARY

The pattern of mitotic activity in the epithelium of the oral mucosa was studied in serial histologic sections obtained from 80 biopsies taken from 54 New Zealand white rabbits 3½ months of age. Twenty-six of these animals were given a single dose of colchicine (1 mg. per kilogram) intraperitoneally. The specimens for biopsy were taken in four groups, six hours apart. The technique and site of biopsy and

21. Brauer.[8] Cowdry.[9]

the histologic preparation and examination of the specimens were kept uniform. For each mitosis, the phase, direction of division when possible, and location were determined. Analysis of the findings led to the following conclusions:

The average mitotic index (the number of cells in mitosis per thousand cells examined) was 5.1 ± 0.29 in normal specimens. At different hours of the day and night the mitotic index ranged from 3.8 ± 0.18 to 7.2 ± 0.56. Mitotic activity showed a slight tendency to cluster in localized areas. Sections from rabbits which had been given colchicine showed that within a period of six hours different areas of the epithelium participated in the mitotic activity. Comparison of the mitotic index in colchicinated specimens with that of normal specimens indicated that the epithelial cells enter mitosis at the rate of 0.48% per hour. The average intermitotic time was estimated to be 208 hours, the average duration of mitosis to be 64 minutes. Sixty per cent of the mitoses in the normal specimens occurred in the basal layer. The remaining 40% were found in the stratum spinosum. The study of planes of cell division in the basal layer indicated that almost half (46.2%) of the daughter cells would immediately become members of the stratum spinosum.

INCREASING INCIDENCE OF ARTERIOSCLEROTIC AORTIC ANEURYSMS

Analysis of Six Thousand Autopsies

ROSARIO MANIGLIA, M.D.
AND
JOHN E. GREGORY, M.D.
PHILADELPHIA

THE HIGH degree of difficulty encountered in correctly diagnosing abdominal aneurysms has been emphasized by a number of clinicians.[1] In Bryant's [2] series of 54 cases only 18 were correctly diagnosed. In 1942 Eliason and McNamee [1b] reported 24 cases, only half of which were correctly diagnosed. Fifty-three different diagnoses were made for the 24 patients. Many of the signs and symptoms suggest renal or perirenal diseases due either to direct pressure on the kidneys or disturbance of the renal blood vessels. Other cases suggest intra-abdominal tumors, such as gastric carcinoma, pancreatic cysts, or retroperitoneal lymphoma. Indeed, abdominal aneurysms have been confused with almost every intra-abdominal and retroperitoneal syndrome known.

It seems apparent that one of the causes for diagnostic confusion has been the supposed rarity of abdominal aneurysms. It is the purpose of this paper to point out that this lesion, while not common, is far from rare and is increasing in incidence.

In 1905 Osler [1a] reported that there had been 16 abdominal aneurysms in the first 18,000 admissions to the Medical Wards of the Johns Hopkins Hospital. In the same group there were 10 times as many thoracic aneurysms. Kampmeier [3] in 1936 reported virtually the same ratio—eight thoracic to one abdominal aneurysm—among the patients of the Charity Hospital in New Orleans. These figures are cited as being generally applicable.[4]

From the Department of Pathology, Hahnemann Medical College.

Read before the Annual Joint Meeting of Section on Clinical Pathology of The Philadelphia County Medical Society and Pathological Society of Philadelphia, March 13, 1952.

1. (*a*) Osler, W.: Aneurysm of the Abdominal Aorta, Lancet **2**:1089, 1905. (*b*) Eliason, E. L., and McNamee, H. G.: Abdominal Aneurysm: Report of 24 Cases, Am. J. Surg. **56**:590, 1942. (*c*) Lipschutz, B., and Chodoff, R. J.: Diagnosis of Ruptured Abdominal Aortic Aneurysms: Report of Case, Arch. Surg. **39**:171, 1939. (*d*) Uhle, C. A. W.: Significance of Aneurysms of Abdominal Aorta Masquerading as Primary Urologic Disease, J. Urol. **45**:13, 1941.

2. Bryant, J. H.: Two Clinical Lectures on Aneurysms of Abdominal Aorta, Clin. J. **23**:71 and 89, 1903.

3. Kampmeier, R. H.: Aneurysms of Abdominal Aorta: Study of 73 Cases, Am. J. M. Sc. **192**:97, 1936.

4. Hall, E. M., in Pathology, edited by W. A. D. Anderson, St. Louis, C. V. Mosby Company, 1948, pp. 589-592.

Our own observations over the last four years led us to question the applicability of these figures at the present time, and thus we undertook to review the literature and to analyze the autopsies performed at Hahnemann Hospital. All dissecting aneurysms have been excluded from this study.

TABLE 1.—*Data on 58,540 Autopsies, Including 962 Aortic Aneurysms*

Author	Year of Report	No. of Autopsies	No. of Aortic Aneurysms	% of Aortic Aneurysms Based on Autopsies	No. of Thoracic Aneurysms	No. of Abdominal Aneurysms	Ratio of Abdominal to Thoracic
Bryant [2]	1906	18,678	325	1.7	271	54	1 to 5
Osler [1a]	1905	2,200	60	2.7	49	11	1 to 4.4
Lucké and Rea [5c]	1921	12,000	244	2.0	204	40	1 to 5.1
Genert [5d]	1923	1,062	28	2.6	22	6	1 to 3.6
Ogden [5e]	1940	9,000	118	1.3	103	15	1 to 6.8
Ruffin and others [5f]	1941	9,000	86	0.9	66	20	1 to 3.3
Present series	1951	6,000	101	1.6	70	31	1 to 2.2
Total		58,540	962	1.6			

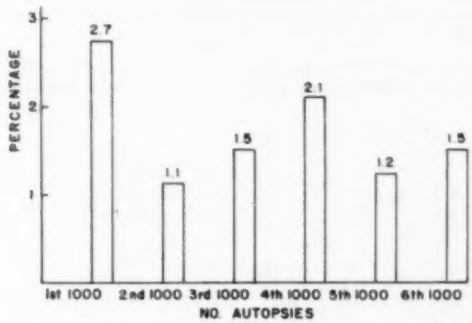
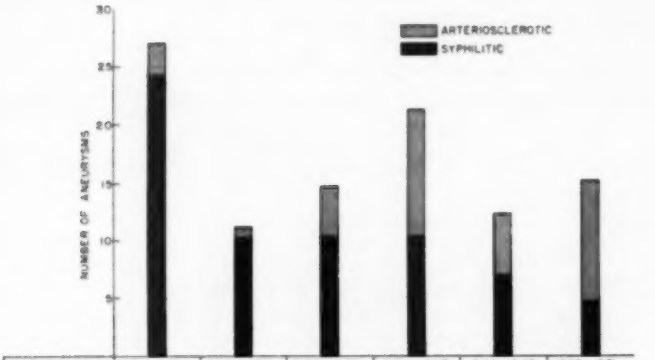


Chart 1.—Percentage incidence of aortic aneurysms per 1,000 autopsies.

In the 6,000 autopsies performed between 1906 and 1952, a diagnosis of aortic aneurysm was made in 101 cases. This is an incidence of 1.6%. This incidence correlates well with the autopsy incidence, shown in Table 1, reported by other authors from 1903 to date.[5] The table includes 58,540 autopsies, among which there were 962 aortic aneurysms, a total incidence of 1.6%. The variation from this average is very small in the individual series. We believe that this indicates that our series is a representative one. It is interesting, and we believe significant, that when percentage incidence of total aneurysms is plotted chronologically per 1,000 autopsies the greatest difference is between 1.1% and 2.7%, and the mean difference is 0.8%.

5. (*a*) Bryant.[2] (*b*) Osler.[1a] (*c*) Lucké, B., and Rea, M. H.: Studies on Aneurysm, J. A. M. A. **77**:935, 1921. (*d*) Genert, E. R.: Abdominal Aortic Aneurysm: Brief Review of 6 Abdominal Aneurysms in 28 Aneurysms Found in 1,062 Autopsies, Kentucky M. J. **21**:405, 1923. (*e*) Ogden, M. A.: Aneurysm of Aorta: Clinico-Pathological Analysis of 127 Necropsies, Urol. & Cutan. Rev. **44**:731, 1940. (*f*) Ruffin, M. de G.; Castleman, B., and White, P. D.: Arteriosclerotic Aneurysms and Senile Ectasia of Thoracic Aorta, Am. Heart J. **22**:458, 1941.



YEARS	1906 - 1931	1932 - 1935	1936 - 1940	1941 - 1945	1946 - 1948	1949 - 1951
NO. OF AUTOPSIES	1 - 1000	1001 - 2000	2001 - 3000	3001 - 4000	4001 - 5000	5001 - 6000
A/S RATIO	1/9	1/10	1/2	1/0.9	1/1.4	1/0.4

Chart 2.—Number of aneurysms and ratio of arteriosclerotic to syphilitic aneurysms per 1,000 autopsies. Black columns indicate syphilitic aneurysms; hatched columns, arteriosclerotic aneurysms.

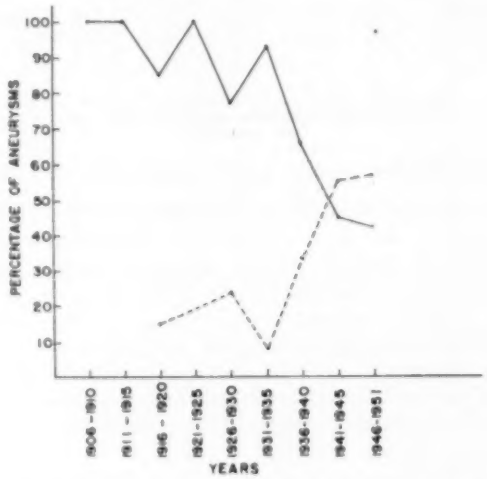


Chart 3.—Changing incidence of arteriosclerotic and syphilitic aneurysms over the years. The solid curve indicates syphilitic aneurysms; the broken curve, arteriosclerotic aneurysms.

Our interest in this subject was originally stimulated by what appeared to us to be an increased number of arteriosclerotic aneurysms. If there has been an actual increase in this type of aortic lesion, and if, as demonstrated, the total incidence has remained virtually constant, then there must have been a decrease in the saccular and fusiform aneurysms which are usually attributed to syphilis. When our series is arranged chronologically per 1,000 autopsies, and divided according to the usual historic, serologic, and histologic criteria into those due to syphilis and those due to arteriosclerosis, an extremely interesting sequence of frequencies is seen. Charts 2 and 3 illustrate these facts. Chart 2 shows that whereas the ratio of arteriosclerotic to syphilitic aneurysms was 1 to 8 in the first 1,000 autopsies, and 1 to 10 in the second, the ratio changed gradually to 1 to 1 in the next 3,000; and in the last 1,000 autopsies there was a reversal of incidence to 1 to 0.4. The same data expressed graphically in Chart 3 show that since 1931 there has been a steady decline in the incidence of syphilitic aortic aneurysms and an increase in the occurrence of arteriosclerotic aneurysms.

TABLE 2.—*Distribution of Aortic Aneurysms As to Site*

				No.	Ratio
Thoracic aneurysms				70	2.2
Etiologic Type	No.	Ratio	%		
Syphilitic	64	10	91		
Arteriosclerotic	6	1	9		
Abdominal aneurysms				31	1
Etiologic Type	No.	Ratio	%		
Syphilitic	1	1	4		
Arteriosclerotic	30	30	96		

It should be stated here that the senile ectasia of Ruffin, Castleman, and White,[37] a cylindrical dilatation and tortuosity of the thoracic aorta, is not considered by us to be an aneurysm, since our definition requires the focal saccular or fusiform enlargement of a segment between relatively normal areas.

The decisions as to etiology were purposely weighted on the side of syphilis. If, from a study of all the available facts of history, serology, and histology, it seemed possible that the lesion was syphilitic in origin, it was so listed.

The same textbook referred to above [4] states that, while syphilis is the major cause of thoracic aneurysms, similar lesions in the abdominal aorta are most frequently due to arteriosclerosis. Analysis of our series as seen in Tables 2 and 3 is in agreement with this generalization. In Table 2 it is shown that of the 70 thoracic aneurysms 64, or 91%, are syphilitic in origin, while 6, or 9%, are arteriosclerotic. There is an almost exact reversal of etiologic percentages in the 31 abdominal aneurysms. Here there was but one aneurysm (4%) due to syphilis, and thirty (96%) due to arteriosclerosis. When the distribution of aneurysms is plotted for smaller anatomical units of the aorta (Table 3), it is evident that syphilis attacks primarily the proximal portion of this organ and arteriosclerosis the distal portion. Despite the fact that this distribution is well known, we have no satisfactory

explanation, either from our own knowledge or from a pointed search of the literature, for this strange and interesting localization of lesions. The problem is under investigation at the present time.

Not all recently reported series of abdominal aneurysms show the same high proportion of arteriosclerosis as the etiologic agent. In 1940 Hubeny and Pollack,[6] in analyzing 48 abdominal aneurysms, found that 75% were due to syphilis. Four years later Pratt-Thomas [7] reported 17 abdominal aneurysms, in 15 of which syphilis was found to be the cause. Also in 1944 Scott [8] was able to study 96 cases, of which 58% were due to syphilitic aortitis. In contrast to these reports our own series of 31 abdominal aneurysms contains only one we can attribute to syphilis. Of the 20 cases reported in 1941 by Ruffin, Castleman and White,[3f] 17 were due to arteriosclerosis. Blakemore [9] recorded the observation in 1947 that of the 32 cases of abdominal aneurysms seen by him, 26 cases were due to arteriosclerosis. The records of the Mayo Clinic, as reported by Estes [10] in 1950, contain 102 abdominal aneurysms, 97 of which were considered to be arteriosclerotic.

TABLE 3.—*Distribution of Syphilitic Aneurysms*

Site	No.	%
Ascending aorta	41	68
Transverse arch	16	25
Descending arch	2	3
Thoracic aorta	5	8
Abdominal aorta	1	1

Distribution of Arteriosclerotic Aneurysms

Site	No.	%
Arch of aorta	3	8.5
Thoracic aorta	3	8.5
Abdominal aorta	30	83

An explanation for these inconsistencies has been suggested by Scott,[8] who stated,

> the incidence of syphilis in the etiology of abdominal aneurysm varies with the prevalence of syphilis in the community served by the respective hospitals. In Kampmeier's series from the Charity Hospital of New Orleans approximately 80 per cent of the 68 patients were of the colored race, in whom it is known that the incidence of syphilis is high. . . .

Hubeny and Pollack,[6] reporting from the Cook County Hospital in Chicago, note that 71% of their patients were Negroes, and in Scott's group 64% were Negroes. Scott further records that as of 1939 the incidence of syphilis in the Negro population of the Eastern Health District of Baltimore, from which most of his patients were drawn, was about 45% for the age group from 35 to 39.

6. Hubeny, M. J., and Pollack, S.: Saccular Abdominal Aortic Aneurysm: Analysis of 48 Cases, Am. J. Roentgenol. **43**:385, 1940.

7. Pratt-Thomas, H. R.: Aneurysm of Abdominal Aorta: Analysis of 17 Cases, J. South Carolina M. A. **40**:251, 1944.

8. Scott, V.: Abdominal Aneurysms: Report of 96 Cases, Am. J. Syph. **28**:682, 1944.

9. Blakemore, A. H.: Clinical Behavior of Arteriosclerotic Aneurysm of Abdominal Aorta: Rational Surgical Therapy, Ann. Surg. **126**:195, 1947.

10. Estes, J. E., Jr.: Abdominal Aortic Aneurysm: Study of 102 Cases, Circulation **2**:258, 1950.

In our own series, 74% of the patients were white, and a similar proportion is found in most of the series reporting a preponderance of sclerotic lesions.

These facts, however, do not explain the major point to be made in this paper, i. e., that there is a decreasing incidence of syphilitic aneurysms and an increasing incidence of arteriosclerotic aneurysms in a hospital population which, while predominantly white, has not changed appreciably in its Negro-white ratio in the period covered by this report.

A review of the literature reveals that the possibility of a changing ratio of syphilitic and arteriosclerotic lesions has engaged the attention of others. For example, Eliason [10] expressed considerable surprise to find that half of the 24 abdominal aneurysms reported by him in 1942 were due to arteriosclerosis.

The first actually to point out this change in incidence of aneurysms was Epstein [11] in 1945, who stated, "It is significant that the ratio of abdominal to thoracic aneurysms has undergone a change from 1 to 10 in 1905 [Osler [18]] . . .

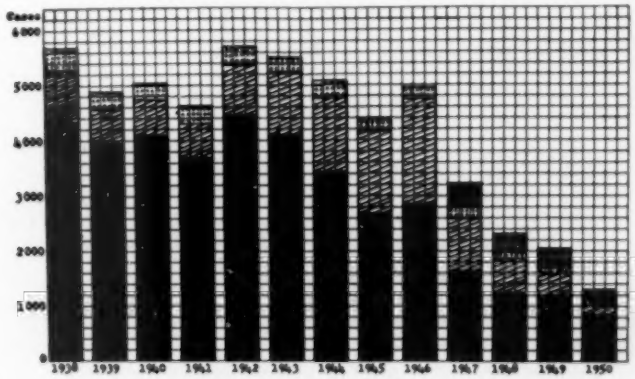


Chart 4.—Decline of syphilis in Massachusetts. Black columns indicate latent and late syphilis; hatched columns, primary and secondary syphilis; stippled columns, congenital syphilis, and cross hatched columns, early latent syphilis. Reprinted from Fiumara,[13] with permission of the author and of the editors of the *New England Journal of Medicine*.

to 1 to 3.3 [Ruffin and co-workers [5f]] in 1941. A declining incidence of aneurysm due to syphilitic aortitis and an increasing incidence of arteriosclerotic aneurysms is suggested by these figures."

Data compiled more recently add further weight to Epstein's suggestion. For example, in 1948 Foord [12] recorded a ratio of abdominal to thoracic aneurysms of 1 to 3 in his material, and, in our own series, through 1951, we found the ratio to be 1 to 2.2. It should be made clear that all the ratios mentioned above include data from the early years of this century, a period when syphilitic aneurysms formed a very high proportion of the total (Chart 3). Since 1949, as shown in Chart 2, we have seen 2.5 times as many arteriosclerotic as syphilitic aortic aneurysms.

11. Epstein, J.: Aneurysms of the Abdominal Aorta, Ann. Int. Med. **22**:252, 1945.

12. Foord, E.: Aneurysm of the Abdominal Aorta, Yale J. Biol. & Med. **20**:441, 1948.

We should like to suggest two major causes for the changes noted in this report. The first of these is the actual declining incidence of syphilis, particularly the

TABLE 4.—*Symptomatic Late Syphilis Reported From Jan. 1, 1945, Through Dec. 31, 1951*

Year	Cardiovascular	C. N. S.
1945	78	185
1946	67	167
1947	48	263
1948	56	417
1949	43	282
1950	37	266
1951	26 *	198 *

* 1951 figure is based on total number of cases reported through Sept. 30, 1951.

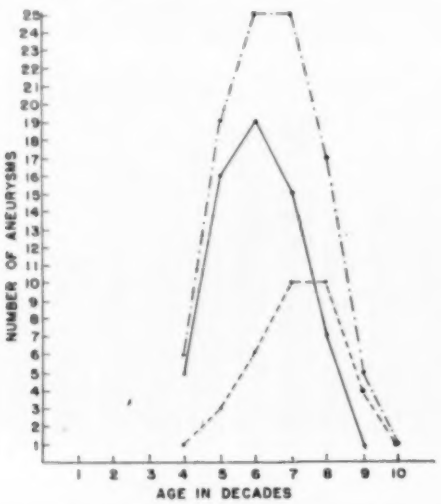


Chart 5.—Age incidence of aortic aneurysms. The upper curve indicates the total number of aortic aneurysms; the middle (solid) curve, the number of syphilitic aneurysms; the bottom (broken) curve, the number of arteriosclerotic aneurysms.

decline in the later stages of the disease. Fiumara [13] has shown, as illustrated in Chart 4 that in Massachusetts the reported incidence of late and latent syphilis has decreased from about 4400 cases per year in 1942, to 800 cases in 1950. Data from the City Public Health Department, obtained through the courtesy of Dr. Norman R. Ingraham,[14] indicates similarly that in Philadelphia, from 1945 through 1951, a period when reporting was at a maximum and constant, that the incidence of syphilis affecting the cardiovascular system was declining. Dr. Ingraham has cautioned that the period recorded is really too short for defining significant trends, but the figures are compatible with the data from Massachusetts. The changes noted

13. Fiumara, N. J.: Congenital Syphilis in Massachusetts, New England J. Med. **245**:634, 1951.

14. Ingraham, N. R.: Personal communication to the authors.

in Table 4 and Chart 4 are probably the result of several factors, including better public health measures, better diagnosis, and more effective therapeutic agents, as well as an increasing awareness of the disease on the part of the general public.

The second major cause is the increasing incidence and severity of arteriosclerosis. It is well known that incidence and severity of arteriosclerosis increase with age.[15] That the general population is increasing in age is also well known. This is supported by data supplied by the Metropolitan Life Insurance Company,[16] which indicate that life expectancy at birth has increased from 48.2 years in 1900, to 65.9 years in 1949. With this tremendous increase in the number of persons in the age groups above 50 years, it is reasonable to expect an increase in the lesions which occur most frequently in the second half century of life. As shown by Chart 5, the peak incidence of arteriosclerotic aneurysms in our series lies between the sixth

TABLE 5.—*Age Distribution*

Age, Years	Arteriosclerotic		Syphilitic		Total
	No.	%	No.	%	No.
31- 40	1	2.8	5	7.9	6
41- 50	3	8.5	16	25	19
51- 60	6	17	19	30	25
61- 70	10	28	15	23	25
71- 80	10	28	7	11	17
81- 90	4	11	1	1.5	5
91-100	1	2.8	..	...	1

and seventh decades, and Table 5 indicates that 88% of the arteriosclerotic aneurysms occurred in patients over 50 years of age.

SUMMARY

In a chronological analysis of 6,000 autopsies from 1906 through 1951, 101 aortic aneurysms were found. Since 1931 there has been a steady decrease in the number of syphilitic aneurysms, whereas the number of arteriosclerotic aneurysms has increased.

In recent years abdominal aneurysms were encountered more frequently than thoracic aneurysms, a finding which is compatible with the shift in etiologic preponderance, since arteriosclerosis affects the abdominal aorta most severely.

Evidence is presented which suggests that the reversal in incidence of the two major causes of aortic aneurysms is due, at least in part, to a decrease in cardiovascular syphilis and an increase in the age of the population.

15. Duff, G. L., and McMillan, G. C.: Pathology of Arteriosclerosis, Am. J. Med. **11**:92, 1951.

16. Progress in Safety, Statis. Bull. Metrop. Life Insur. Co. (No. 2) **30**:2, 1949; Supplement, 1951.

Case Reports

BILATERAL APLASIA OF THE LUNG

PETER E. TUYNMAN, M.D.
AND
LAWRENCE WILLIAM GARDNER, M.D.
DETROIT

THE OCCURRENCE of agenesis of bronchopulmonary structures is rare, whether the condition is diagnosed during life or discovered at the necropsy table. For the purpose of clarification, we will adhere to the classification proposed by Schneider,[1] which has been cited by many authors[2]; (*a*) true agenesis, in which there is absence of lung, bronchus, and vascular structures on the affected side; (*b*) aplasia of lung, in which the trachea possesses small rudimentary bronchi but pulmonary and vascular structures are absent.

An exhaustive review of the world's literature on pulmonary agenesis was presented by Smart[3] in 1946, supplemented by two cases of his own observation. This author compiled a total of 106 reported cases of unilateral congenital aplasia of the lung. Of these, correct diagnoses were made in 77 cases, and the remaining 29 were discarded for various reasons. Of the 77 cases, absence of the left lung was reported in 45 and of the right lung in 29; in the remaining 3 cases, the side of the absent lung was not indicated. Complete absence (agenesis) of a lung occurred in 33 cases, in 18 of which agenesis was on the left and in 15 on the right side. In 35 cases, a rudimentary bronchus (aplasia) or rudimentary lung (hypoplasia) was present. Of this group, the defect was on the left side in 23 cases, and on the right side in 12 cases. In the remaining cases Smart was unable to find an accurate record.

This condition occurred in males 27 times and in females 35 times; the sex of the remaining group was not stated. Thirty-five of the patients in the reported cases were children under one year of age. The oldest patient was a 72-year-old man.

From the Division of Laboratories, Mount Carmel Mercy Hospital.

1. Schneider, P.: Die Missbildungen der Atmungsorgane, in Schwalbe, E.: Die Morphologie der Missbildungen des Menschen und der Tiere, Jena, Gustav Fischer, 1900-1913, Vol. 3, Pt. 2, p. 772.

2. (*a*) Hurwitz, S., and Stephens, H. B.: Agenesis of Lung: Review of Literature and Report of Case, Am. J. M. Sc. **193**:81-87 (Jan.) 1937. (*b*) Olcott, C. T., and Dooley, S. W.: Agenesis of Lung in Infant, Am. J. Dis. Child. **65**:776-780 (May) 1943. (*c*) Valle, A. R., and Graham, E. A.: Agenesis of Lung, J. Thoracic Surg. **13**:345-356 (Aug.) 1944. (*d*) Ferguson, C. F., and Neuhauser, E. B. D.: Congenital Absence of Lung (Agenesis) and Other Anomalies of Tracheobronchial Tree, Am. J. Roentgenol. **52**:459-471 (Nov.) 1944. (*e*) Field, C. E.: Pulmonary Agenesis and Hypoplasia, Arch. Dis. Childhood **21**:61-75 (June) 1946.

3. Smart, J.: Complete Congenital Agenesis of a Lung, Quart. J. Med. **15**:125-139 (April) 1946.

Since Smart's comprehensive review, additional cases have been recorded by Widerman and Peters,[4] and Nesbit, Paul and Middleton.[5] It is therefore clearly evident that congenital aplasia or agenesis of one lung is a rare condition.

Congenital absence of both lungs, though of less clinical significance, is a much rarer phenomenon and, of course, incompatible with life. Only two cases have been recorded in the literature, one by Schmit [6] in 1893 and the other by Allen and Affelbach [7] in 1925. The rarity of this condition prompts the following detailed report.

REPORT OF A CASE

The mother of this infant was a 25-year-old white woman of apparently good health and in her second pregnancy. The first pregnancy terminated at the 12th week of gestation as a spontaneous abortion; the fetus was not examined. One year later the second pregnancy came to full term. The mother had had no previous full-term pregnancies. Her last menstrual period had occurred Feb. 17, 1948. Quickening had been felt on July 3. The entire period of gestation had been uneventful. The mother had not experienced any illness, infection, or dietary deficiencies. She had gained 25 lb. (11.3 kg.) in weight. Examination during labor revealed a vertex presentation, left occiput anterior position, and fetal heart rate of approximately 130 per minute. Analgesia was obtained by means of pudendal block. Delivery was assisted by the use of low forceps at 9 a. m. on Nov. 28, 1948. Upon delivery, the infant appeared well developed. However, normal respiratory activity did not ensue, although the chest was observed to move several times as if respiration were being attempted. Examination revealed good cardiac activity. Respiratory obstruction was suspected, and an intrathecal catheter was immediately inserted. Aspiration was performed, and the administration of oxygen was instituted under positive pressure through the catheter. It was then noted that with each administration of oxygen the upper abdomen became distended. However, no expansion of the chest resulted from this procedure. The presence of a tracheoesophageal fistula with resultant transmission of oxygen into the esophagus and stomach was entertained. Artificial respiration was given for 45 minutes, during which time no voluntary respiratory activity occurred. Cardiac activity gradually weakened and ceased at 9:45 a. m.

Autopsy Findings.—The body was that of a newborn male infant measuring 62 cm. in length and weighing 6 lb. 13 oz. (3,090 gm.). Externally, the infant was well developed, and no deformities or malformations were observed. The scalp was covered by a moderate amount of dark brown hair. The fontanels were neither depressed nor elevated and were of normal configuration. Results of examination of the eyes, ears, nose, mouth, and neck were not remarkable. The chest was normal in size and dull upon percussion. The abdomen was flat, and the liver edge was slightly palpable beneath the right costal margin. The anus was perforate. The upper and lower extremities were symmetrical and proportionate. Jaundice and edema were not seen.

When the peritoneal cavity was opened, the liver edge was noted to extend 0.8 cm. below the right costal margin at the midclavicular line. The diaphragm on the left side was at the level of the sixth rib; on the right side its height could not be ascertained from the peritoneal aspect.

After removal of the sternal plate, it was apparent that the upper portion of the left pleural cavity was unoccupied space. The pericardial sac was readily observed in the lower half of

4. Widerman, A., and Peters, C. H.: Agenesis of Lung: Case Report, J. Pediat. **28**:204-206 (Feb.) 1946.

5. Nesbit, W. M.; Paul, L. W., and Middleton, W. S.: Congenital Aplasia of Lung: Case Report, Am. J. Roentgenol. **57**:446-448 (April) 1947.

6. Schmit, H.: Ein Fall von vollständiger Agenesie beider Lungen, Arch. path. Anat. **134**:25-32 (Oct.) 1893.

7. Allen and Affelbach: Congenital Absence of Both Lungs, Surg., Gynec. & Obst. **41**:375-376 (Sept.) 1925.

the left chest. The pericardium was adherent to the left lateral chest wall from the level of the third interspace to the costophrenic angle. Pulmonary tissue or bronchial structure was not evident.

Examination of the right hemithorax revealed a firm mass which extended to the first intercostal space. Inspection and palpation first suggested that this mass was lung tissue of greatly increased consistency. However, careful observation and dissection disclosed that it represented a projection from the right lobe of the liver upward into the right chest to the level of the first intercostal space. This anomalous portion of hepatic tissue was covered by the right leaf of the diaphragm, which was extremely thin. The right lung was absent.

The glottis, epiglottis, larynx, thyroid gland, parathyroid glands, and thymus were well-formed structures with the usual anatomic relationships.

The trachea was dissected and removed in its entirety. On examination, a small bud was found to be present on the left side of the trachea near its inferior end, and a slightly larger bud was present on the right side. These bronchial buds were the end-point of pulmonary

Fig. 1.—Posterior aspect of larynx and trachea with a minute left bronchial bud and a longer right bronchial bud; absence of pulmonary tissue.

development in this infant. The trachea was well developed and contained 14 cartilaginous rings; the lumen was uniform in caliber and measured 0.7 cm. in diameter.

The left lung bud was noted 2.6 cm. below the cricoid cartilage. This primitive structure measured 0.5 cm. in diameter and protruded 0.2 cm. in a posterolateral direction from the external surface of the tracheal wall. The right lung bud was slightly larger and extended 1.2 cm. below the left bud in a similar direction. The left lung bud presented a rather blunt contour, whereas the right was more acute in its termination. Apparently, these structures represented the right and left bronchi (Fig. 1).

The larynx and trachea were opened longitudinally on the posterior surface. Examination of the anterior failed to disclose obstructions or local lesions. The mucosal lining was yellowish-gray and smooth. A small pocket extended into the left lung bud, measuring 2 mm. in diameter and 2 mm. in depth. The longitudinal excision was continued to the tip of the right lung bud. From the trachea, there was a gradual diminution in size, ending in complete impatency at the tip of the lung bud (Fig. 2). Close examination revealed without a doubt that no branching

Fig. 2.—Opened trachea with arrow indicating left bronchial bud above cone-shaped right bronchial bud; absence of pulmonary tissue.

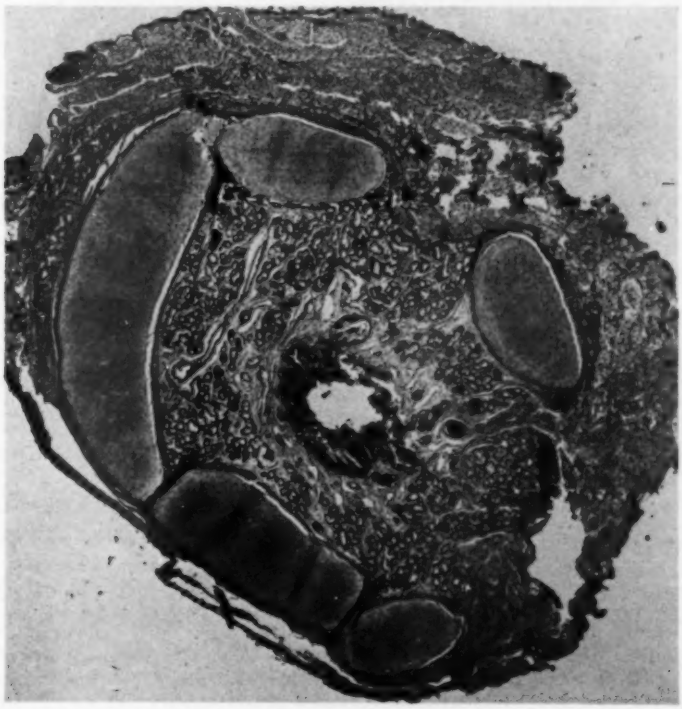


Fig. 3.—Section from near tip of left bronchial bud, showing minute lumen surrounded by mucous glands; complete absence of pulmonary tissue.

structures or pulmonary tissue was present. Therefore, with the exception of the above described lung buds, there was complete absence of both lungs.

Prior to removal of the heart, the great vessels were carefully dissected in search of anomalies. The ascending aorta was in its usual location. However, the arch coursed to the right and from this portion arose the innominate, right common carotid, and right subclavian arteries. The descending segment was on the right, posterior to the esophagus. Careful examination failed to reveal bronchial arteries, but the intercostal vessels were well developed.

The heart measured 2.4 cm. in its greatest transverse diameter and weighed 12 gm. The right auricle was slightly dilated and received only the superior vena cava. The inferior vena cava did not enter this chamber, as will be described below. The fossa ovalis was not patent, and no other interauricular defect was seen. The tricuspid valve presented a normal anatomic

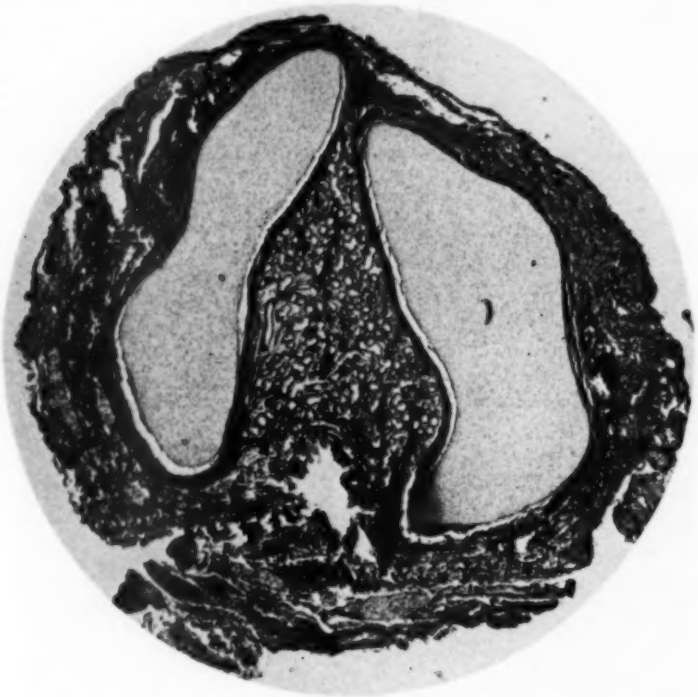


Fig. 4.—Section from tip of right bronchial bud, showing two masses of hyaline cartilage separated by mucous glands embedded in connective tissue; no evidence of a bronchial lumen or pulmonary tissue.

appearance. The right ventricle was slightly dilated. The pulmonary valve was well formed. The pulmonary artery was opened in a linear fashion. At a distance of 1.8 cm. from the pulmonary valve, it entered directly into the aorta, at a point just distal to the origin of the right subclavian artery, the usual site of the ductus arteriosus. The pulmonary artery itself had no branches and was patent through its short course. The pulmonary artery at its origin was 5 mm. in diameter and 4 mm. at the point of entrance into the aorta.

The left auricle was a relatively small chamber, receiving but one vessel instead of the usual four pulmonary veins, which were absent. The one vessel which entered the left auricle was the anomalous inferior vena cava. This vessel had been carefully dissected from below upward

and found to enter directly into the lower portion of the left auricle. It measured 2 mm. in diameter. No abnormalities of the mitral valves were noted. Evidence of an interventricular defect was not found. As indicated above, the aorta was anomalous in that the arch extended to the right and the descending portion of the thoracic segment lay in the right side of the mediastinum.

The liver weighed 120 gm. An unusual thumb-shaped projection extended upward from the right lobe of the liver to the level of the first intercostal space. It would appear that this development was an outward growth following the course of lessened resistance caused by the absence of the right lung. Serial section of the liver revealed a medium-brown cut surface with an ill-defined lobular pattern.

Comparison of Reported Cases of Bilateral Aplasia of the Lung

	I. Schmit	II. Allen and Affelbach	III. Present Case
Gestation	8 months	Term	Term
Sex	Not stated	Female	Male
Length	44.5 cm.	47 cm.	52 cm.
Weight	Not stated	5 lb. 2 oz.	6 lb. 13 oz.
Trachea	10 rings; connected with esophagus for 18 cm.	Normal	14 rings
Bronchi	Absent	Primitive buds	Primitive buds
Pleural cavities	Absent	Absent	Absent
Pulmonary tissue	Absent	Absent	Absent
Esophagus	Tracheal fusion	Normal	Normal
Pericardial sac	Displaced to right	Large; extended to each lateral chest wall	Displaced to the left
Heart	4 chambers	4 chambers; 5 cm. in greatest transverse diameter	4 chambers; 2.4 cm. in greatest transverse diameter
Superior vena cava	Not described	Not described	Normal
Inferior vena cava	Not described	Not described	Entered left auricle
Right auricle	Not described	Not described	Slightly dilated
Foramen ovale	Patent	Patent (6 mm.)	Anatomically closed
Right ventricle	Not described	Not described	Slightly dilated
Pulmonary artery	Entered aorta	Entered aorta	Entered aorta
Pulmonary veins	Absent	Absent	Absent
Left auricle	Received no vessels	Received no vessels	Received inferior vena cava
Left ventricle	Not described	Not described	Normal
Aorta	Normal	Normal	Right-sided arch and descending segment
Liver	Small accessory lobe (left)	Normal	Extended into right chest
Diaphragm	4th rib on left 8th rib on right	4th rib on right and left	1st intercostal space, right; 6th rib on left
Other anomalies	Osseous abnormality of neck	None	Accessory spleen

An accessory spleen measuring 0.7 cm. in diameter was located near the tail of the pancreas.

Examination of the remaining structures of the thorax and abdomen, the brain and spinal cord, was not enlightening.

Microscopic Pathology.—At its most inferior end, each bronchial bud consisted of a solid core of mucus-producing glands embedded in a delicate connective tissue framework partially surrounded by irregular masses of hyaline cartilage. At the periphery of the cartilage, a small amount of pink-staining fibrous tissue was noted, in which there was a small amount of fetal fat and occasional cross sections of nerve (Figs. 3 and 4). Parenchymal pulmonary tissue was completely absent. Microscopic examination of the remaining tissues was not remarkable.

Final Diagnoses.—The final diagnoses were (1) bilateral aplasia of lung, and (2) congenital heart disease: (*a*) anomalous aorta with right descending aorta,

(*b*) anomalous inferior vena cava entering in left auricle, and (*c*) pulmonary artery opening into the aorta at usual site of the ductus arteriosus.

COMMENT

Pertinent findings of our case may be compared with those of the previous reported cases shown in the Table.

In the embryological development of the lung, the primary bronchi appear at the 4 mm. stage in the human embryo. At this point, the respiratory tract is represented by a laryngeal slit, a tubular trachea, and two primary bronchi. Soon after the appearance of the bronchi, the right bronchus becomes somewhat larger than the left and is directed more caudad. It would appear that development of the respiratory organs was arrested at this precise point in the case presented. The trachea and primary bronchi are very similar to those depicted at the 5 mm. stage by Arey.[8] It is evident that the development of the respiratory organs has ceased prior to the 7 mm. stage, at which point lateral branching of the primary bronchi occurs. Therefore, it is felt that the interruption of embryological development occurred between the fourth and fifth week of fetal life in this case.

The various anomalies of the great vessels and heart and the upward development of the right lobe of the liver into the vacant right hemithorax were undoubtedly direct results of pulmonary aplasia. It is plausible that these secondary anomalies were of a somewhat compensatory effect necessitated by the absence of the lungs.

The causative factors of embryologic anomalies are for the most part theoretical and, needless to say, controversial. Intrinsic and extrinsic theories have been carefully studied by the embryologists. In a review of the literature on pulmonary agenesis and hypoplasia of the lung, several theories were repeatedly cited. Schneider,[1] noted by Schwalbe, believed that these defects were produced by an inherent deficiency in the germ plasm. Müller [9] offered the theory of external pressure, which is one of the older concepts. The supposition that vascular anomalies may precede the absence of the lung was given by Ellis.[10] Tichomiroff [11] suggested that hydrops fetalis or an early uterine infection may have been the etiologic basis.

More recently, a report upon the relationship of congenital malformations to maternal rubella was published by Miller.[12] Although no specific malformations of the respiratory organs were mentioned, it is plausible that they likewise may be caused by this viral infection.

Hamilton, Boyd, and Mossman [13] feel that most developmental abnormalities can be attributed to genetic causes, which may be mutations or the result of the

8. Arey, L. B.: Developmental Anatomy: Ed. 4, Philadelphia, W. B. Saunders Company, 1940.

9. Müller, H., in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by F. Henke and O. Lubarsch, Berlin, Springer-Verlag, 1928. Vol. 3, Pt. 1, pp. 539-548.

10. Ellis, A. G.: Congenital Absence of Lung, Am. J. M. Sc. **154**:33 (July) 1917.

11. Tichomiroff, M.: Ein Fall von congenitalem Mangel der linken Lunge mit Persistenz der linken oberen Hohlvene bei einem erwachsennen Menschen, Internat. Monatsschr. Anat. u. Physiol. **12**:24 and 37, 1895.

12. Miller, H. C.: The Relation of Maternal Infection to Congenital Malformations of the Heart, Mod. Concepts Cardiovas. Dis. **17**:27-28 (Dec.) 1948.

action of genes. It is their belief that an abnormality is a result due partly to interference with the particular developmental process and partly to the attempts of other processes to compensate for the deficiency. Possibly the cardiovascular and hepatic abnormalities in our case could be described as attempts to compensate for the pulmonary deficiencies. The role of the genes may be the production of chemical substances, which possess the dual function of affecting differentiation and the affecting rate of developmental changes. Malformations may result from asynchronization of the timing of developmental processes.

SUMMARY

We are presenting the third reported case of bilateral aplasia of the lung associated with congenital heart disease in the form of an anomalous aorta with right descending aorta, anomalous inferior vena cava entering the left auricle, and pulmonary artery opening into the aorta at the usual site of the ductus arteriosus. The pertinent data of this case are compared with those of the previously published cases.

13. Hamilton, W. J.; Boyd, J. D., and Mossman, H. W.: Human Embryology, Baltimore, Williams & Wilkins Company, 1945.

METASTATIC CYSTADENOCARCINOMA OF OVARY THIRTY-THREE YEARS AFTER REMOVAL OF PRIMARY GROWTH

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NEW YORK

IN THE case now to be reported, metastatic serous cystadenocarcinoma of the ovary caused death some 33 years after removal of the primary growth, without having given rise to symptoms during the interim. The case provides an extreme example of the ability of cancer cells to lie latent for long periods and then to grow again.

REPORT OF CASE

A 62-year-old, white, married, French woman was admitted to the New York Hospital with the chief complaint of "swelling of the right leg" of six months' duration. During the same period she had also noticed intermittent abdominal swelling, dysuria, and increasing constipation. Her stools had been unusually dark and occasionally had contained small masses of "tissue." She had gained 20 lb. (9 kg.) in the preceding four months.

Thirty-three years previously, an exploratory laparotomy had been performed at the Roosevelt Hospital, New York, because of a protuberant abdomen and irregular menses. Dr. Madison B. Brown and Dr. Walter W. Brandes of that hospital have generously provided data and materials for inclusion in this report. An ovarian cyst was found on the right side; it measured 13 cm. across and was covered by a cauliflower-like growth over one surface. The opposite ovary was not enlarged but was cystic and presented a similar cauliflower-like growth over one pole. Bilateral salpingo-oophorectomy, including removal of the right-sided ovarian cyst, total hysterectomy, appendectomy, and regional lymphadenectomy were performed. The histologic diagnosis was bilateral papillary cystadenoma of the ovary, chronic appendicitis, and hyperplasia of the lymph node. The uterus and fallopian tubes were unremarkable. No postoperative x-irradiation was administered. Following the operation, the patient remained asymptomatic for 33 years. Six months before her death, edema of the right lower extremity developed, with abdominal swelling and constipation.

Physical examination at the time the patient was admitted to the New York Hospital revealed an obese woman with a firm, fixed mass occupying the lateral half of the right lower quadrant of the abdomen and massive edema of the right lower extremity. The blood (chemically examined) and the urine were unremarkable; a barium sulfate enema revealed partial obstruction due to a neoplasm of the cecum, and intravenous pyelography demonstrated a nonfunctioning right kidney. Ileotransverse colostomy was performed, and the patient died three days postoperatively.

At postmortem examination a broad, grayish, honeycombed, extremely fibrotic and partially necrotic mass of tumor tissue was found to occupy the right hemipelvis and iliac region. It invaded the right ilium and extended from the anterior-superior iliac spine along the anterior border of the ilium to invade the right pubic bone and surround the femoral vessels. The tumor mass was 4 cm. in thickness and extended 8 cm. medially to infiltrate the serosal surface of the cecum. In one area the tumor extended through the wall of the cecum to project from the mucosal surface as a 2-cm. intraluminal polypoidal mass. The distal third of the right ureter was surrounded and compressed by the tumor, with hydroureter and hydronephrosis as a result. A single metastatic nodule measuring 1.5 by 1 by 1 cm. extended into the liver from

From the Department of Pathology of the New York Hospital—Cornell Medical Center.

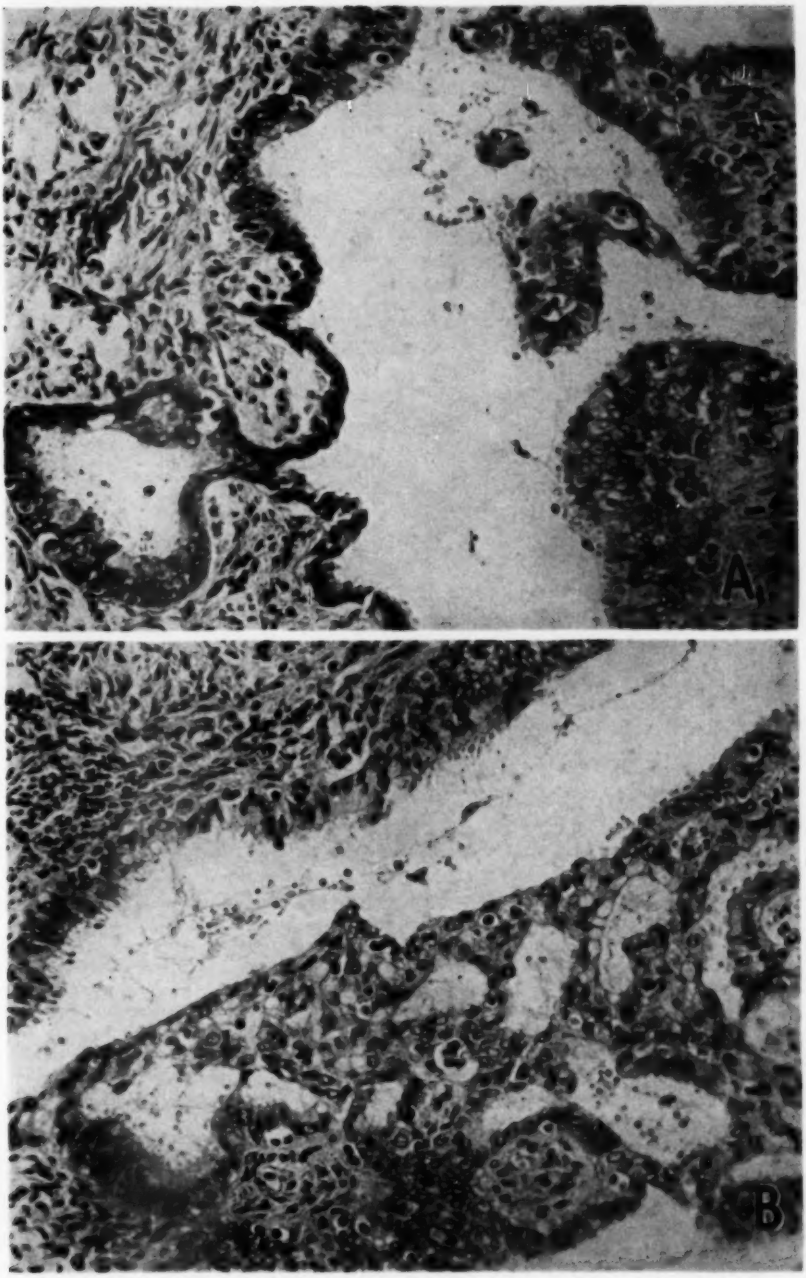

Fig. 1.—Tumor of the ovary removed 33 years before death: *A*, slight cellular anaplasia and multilayering of the epithelium lining the cysts; *B*, intracystic papillary processes; hematoxylin and eosin; × 240.

the peritoneal surface of the right lobe. The lymph nodes along the iliac, aortic, and mesenteric vessels were enlarged and contained metastatic tumor. Careful search failed to disclose a primary tumor elsewhere during the course of a complete autopsy. The only other abnormality of interest was a noninflamed Meckel's diverticulum, 4 cm. in length, located 65 cm. proximal to the ileocecal junction.

Histologically, the main tumor mass was composed of cysts varying from 1 to 12 mm. in diameter, with broad walls of dense fibrous tissue. The cysts were lined by single or stratified cuboidal epithelial cells exhibiting a moderate degree of anaplasia (Fig. 3*A*). Their nuclei showed moderate variation in size and shape and peripheral condensation and central clumping of chromatin; only a thin rim of cytoplasm surrounded the nuclei. Mitosis was infrequent. Occasional well-differentiated cells exhibited elongated processes resembling cilia. Many thin-to-thick, anastomosing, fibrovascular, papillary processes projected into the cysts and were covered by the same type of epithelium. Small groups of tumor cells invaded the fibrous walls in many places. Psammoma bodies were numerous within the cysts and in their walls. Histo-

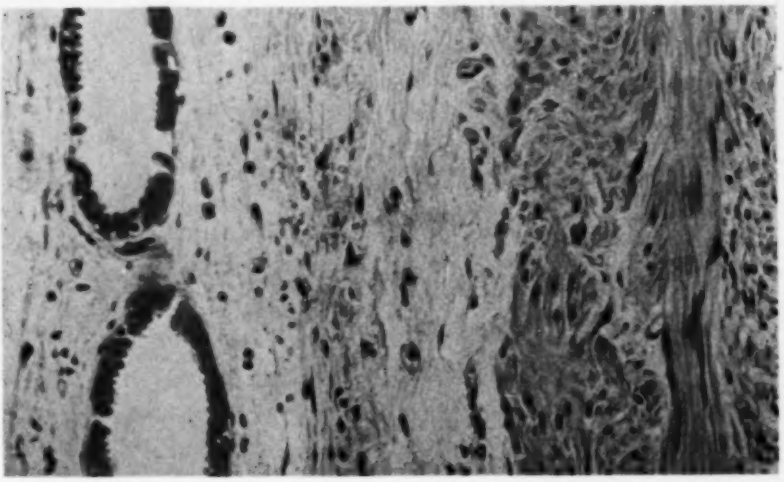

Fig. 2.—Epithelial acini in the subserosa of the appendix removed along with the pelvic organs 33 years before death; hematoxylin and eosin; × 240.

logically, the metastatic foci in lymph nodes (Fig. 3*B*), liver, cecum, and ilium (Fig. 3*C*) resembled the large tumor, though the connective tissue component was less abundant.

Microscopic sections from the operative specimens removed 33 years previously were reviewed and the original growth was compared with that of the metastatic tumor. The ovarian cysts were lined by a layer of cuboidal-to-columnar epithelial cells which displayed multilayering, adhesion of intracystic papillary processes, and slight anaplasia (Fig. 1*A* and *B*). Mitosis was rarely encountered. Many of the cells lining the cystic spaces were heavily ciliated. Psammoma bodies were present but were not so numerous as in the tumor at the time of death. Two acini lined by a single layer of ciliated, tall cuboidal cells with enlarged nuclei were discovered in the subserosa of a section through the appendix (Fig. 2). These were surrounded by unaltered

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EXPLANATION OF FIGURE 3

Fig. 3.—Metastatic tumor present at autopsy: *A*, anaplasia and multilayering of the cells of the main tumor mass in the pelvis; *B*, para-aortic lymph node metastasis, in which psammoma bodies are present within the glandular spaces; *C*, metastatic tumor in the ilium; hematoxylin and eosin; × 240.

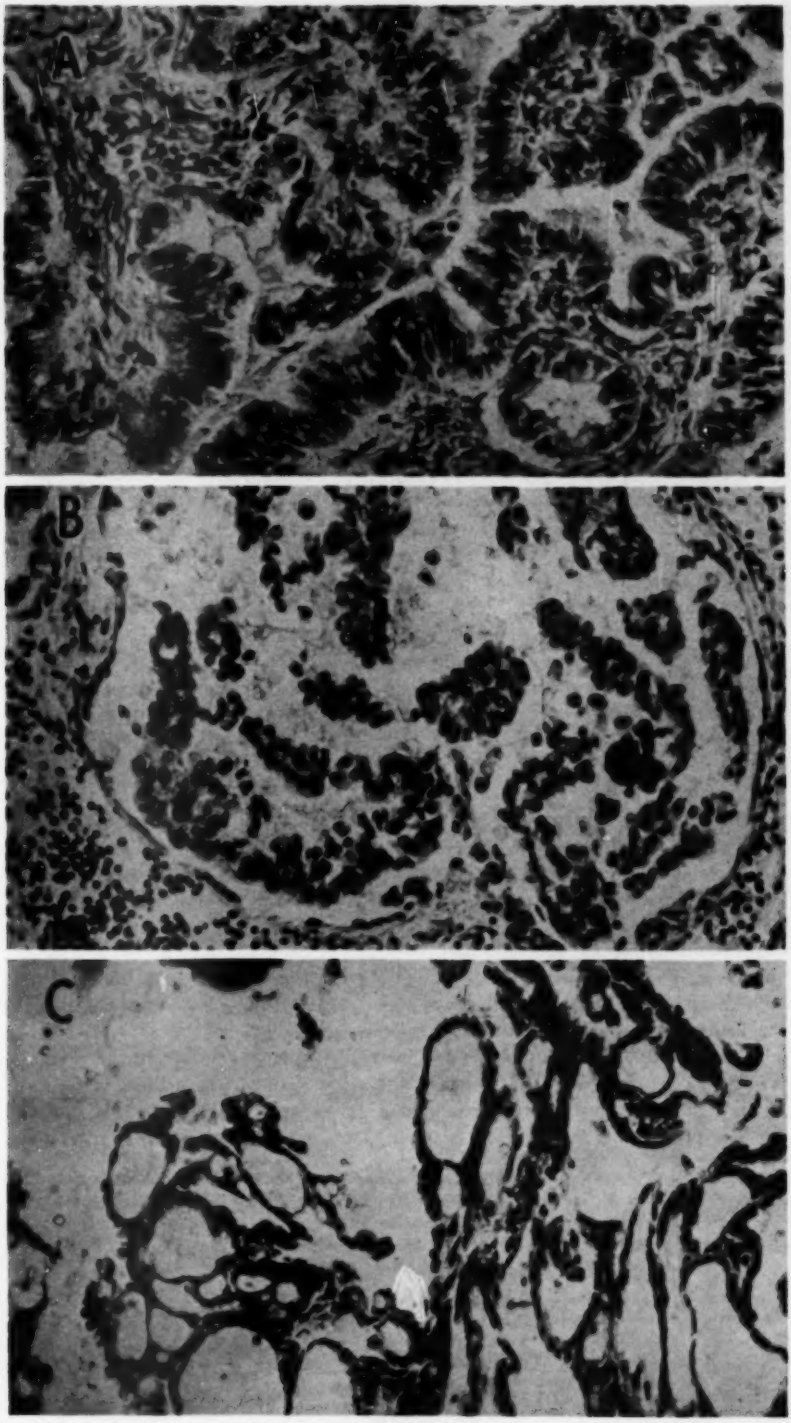

Figure 3

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subserosal connective tissue. The malignant nature of the primary tumors was manifested by the multilayering of the epithelium lining the cysts, the apparent stromal invasion, the anaplasia of the constituent cells, and the intracystic papillary adhesions. The nature of the epithelial acini in the subserosa of the appendix is difficult to determine, but in the absence of a stromal reaction about the acini or other evidence of endometriosis it is probable that they represent metastases from the ovarian tumor.

The original and the metastatic tumors were interpreted as papillary serous cystadenocarcinomas exhibiting low and high histologic grades of malignancy, respectively.

COMMENT

As is well known, papillary serous cystadenocarcinoma of the ovary is generally associated with a poor prognosis. The five-year survival percentages vary from 0.0% in Stout's series,[1] to 30.4% and 39% in those of Munnell and Taylor[2] and Pemberton,[3] respectively. It is evident, however, that some such growths with a low histologic grade of malignancy may survive for a long period of time. In Pemberton's series, for example, three such patients survived 5, 9, and 15 years, with residual cancer following removal of the original growths,[3] and Munnell and Taylor have reported 13 cases in which the patients survived five years or more following the removal of cystadenocarcinoma of low histologic malignancy from one or both ovaries.[2] One of the patients died of recurrent carcinoma 14 years after the original diagnosis was made. The literature of clinical cancer, particularly that of cancer of the breast,[4] likewise discloses numerous examples of death from recurrent or metastatic cancer many years after removal of the primary growth, while the ability of cancer cells to lie latent in tissues has also been observed under experimental conditions.[5] In few or none of the instances studied thus far, however, has the latent period been so long as in the present case.

SUMMARY

A case is reported of a 62-year-old woman who had papillary cystadenocarcinomas of both ovaries removed 33 years prior to death. She remained asymptomatic until six months before death when edema of the right lower extremity, swelling of the abdomen, and constipation developed. At postmortem examination a large, dense, honeycombed tumor was found occupying the right pelvic and iliac regions and extending into the iliac bone and cecum. The liver and iliac, aortic, and mesenteric lymph nodes contained metastatic tumor. Histologically, the tumor represented papillary cystadenocarcinoma similar to but more anaplastic than the growths originally removed.

The case is remarkable for the longest recorded period of survival of a patient with this type of ovarian carcinoma in whom the growth was present at the time of death; it provides an extreme example of the ability of cancer cells to lie dormant for long periods and then to grow again.

1. Stout, A. P.: Human Cancer, Philadelphia, Lea & Febiger, 1932.
2. Munnell, E. W., and Taylor, H. C.: Am. J. Obst. & Gynec. **58**:943, 1949.
3. Pemberton, F. A.: Am. J. Obst. & Gynec. **40**:751, 1940.
4. Head, J. R.: Surg., Gynec. & Obst. **44**:262, 1927.
5. Gardner, W. U.: Cancer Res. **5**:497, 1945.

Books

POISONING: A GUIDE TO CLINICAL DIAGNOSIS AND TREATMENT. By W. F. von Oettingen, M.D., Ph.D., National Institutes of Health, U. S. Public Health Service, Federal Security Agency, Bethesda, Md. Price, $10.00. Pp. 524. Paul B. Hoeber, Inc., 49 E. 33d St., New York 16, 1952.

The author states that the "purpose of this book is to organize today's knowledge of the subject in a fashion which will most readily aid the general practitioner and internist in diagnosis and treatment of poisons." To do this he has divided the material into four sections:

1. The "Introduction" includes classification and diagnosis of poisonings, medicolegal responsibilities, and emergency methods and equipment. This section loses its impact in that it is very brief and, as might be expected from the author's background, more concerned with occupational poisoning than with poisoning of nonindustrial origin.

2. In the second section (designated "Diagnosis," and including "Structural and Functional Pathology") numerous lists of poisons are given under headings of objective or subjective symptoms. While these are grouped under organ systems, it is first necessary to use the index (which is adequate) to locate the particular clinical finding. The lists are quite voluminous in many cases—e. g., some seventy-four substances are listed under coma; but, while such things as chokecherry and richweed are listed, the reader fails to find opium or any of its alkaloids.

3. In the section designated "Management" are included qualitative tests for some 69 poisons. Very many sections begin with "According to . . . ," which gives the impression that the author scanned the literature but has not used the tests personally. Surely the heading "Iodine" should be "Iodide." While some of the tests are within the scope of a hospital laboratory, it is evident that many are quite technical and require special apparatus. Assuredly, the carrying-out and interpretation of the tests are not so simple and final as one may believe from the reading. Strangely, no mention is made of analysis of vomitus or gastric washings, which in toxicological practice, are often the only specimens available. While they may not be of ultimate forensic value, they should be considered from the clinical aspect. Numerous references are given for quantitative determinations.

4. This section is concerned with treatment. After a brief consideration of rationale, removal and elimination of toxic agents, detoxification [which is actually a very short consideration of antidotes], symptomatic and general treatment, 461 different poisons, arranged in alphabetical order, are considered and treatment outlined. The alphabetical arrangement does not follow either official or commercial names; hence it is necessary to use the index in many cases. For example, benadryl® and benzedrine® are so listed, but alcohol is under ethanol. While this term may be the preferred one, it is not the one most commonly used. No mention is made of antabuse® in the book. Typographical errors are few, and the print is easy to read. The author seems to be on more solid ground when considering occupational poisons. Although the book impresses the reviewer as more of a compilation of the literature than material of firsthand information, the author has accomplished to a reasonable degree his purpose of providing a quick reference book of value to clinicians. It has a modicum of directive use for pathologists and toxicologists in that the poisons which have been reported to effect pathologic changes are grouped under each condition.

BASIC PRINCIPLES OF CANCER PRACTICE: A BOOK ON DIAGNOSIS, PROGNOSIS, AND TREATMENT OF HUMAN NEOPLASMS FOR THE GENERAL PRACTITIONER AND MEDICAL STUDENT. By Anderson Nettleship, M.D., F.C.A.P.; Fellow, New York Academy of Medicine; Professor of Pathology, University of Arkansas School of Medicine; Pathologist-in-Chief, University Hospitals, Little Rock, Ark. Price, $7.00. Pp. 398, with 106 illustrations. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 6, 1952.

Because too much of cancer literature is written by and for specialists, the author saw a need to consider the subject "broadly," in order "to guide the relatively inexperienced and refresh the physician." The volume is designed for the general practitioner and medical student. It

takes up certain general principles of tumor biology, including methods of diagnosis, from the clinical-pathological standpoint and then considers in moderate detail the neoplasms of the different organ systems. The book is not designed for, and cannot be recommended to, pathologists. For the general practitioner who has been out of touch with academic medicine, or with recent developments, the volume has considerable valuable material.

A Textbook of Pathology. By E. T. Bell, M.D., Emeritus Professor of Pathology in the University of Minnesota. Contributors: B. J. Clawson, M.D., Emeritus Professor of Pathology in the University of Minnesota, and J. S. McCartney, M.D., Professor of Pathology in the University of Minnesota. Seventh edition. Price, $12.00. Pp. 1008, with 529 illustrations and 5 plates in color. Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, 1952.

The seventh edition of this standard and well-known text appears five years after the sixth edition. The general format is retained, but numerous changes have been made. The volume is almost 100 pages longer, and there are 529 illustrations, a net increase of 29. Some of the older pictures have been replaced or omitted. There is a moderate revision of references. The quality of illustrations, the paper, and the print are excellent. The book, as always, is a highly satisfactory text.

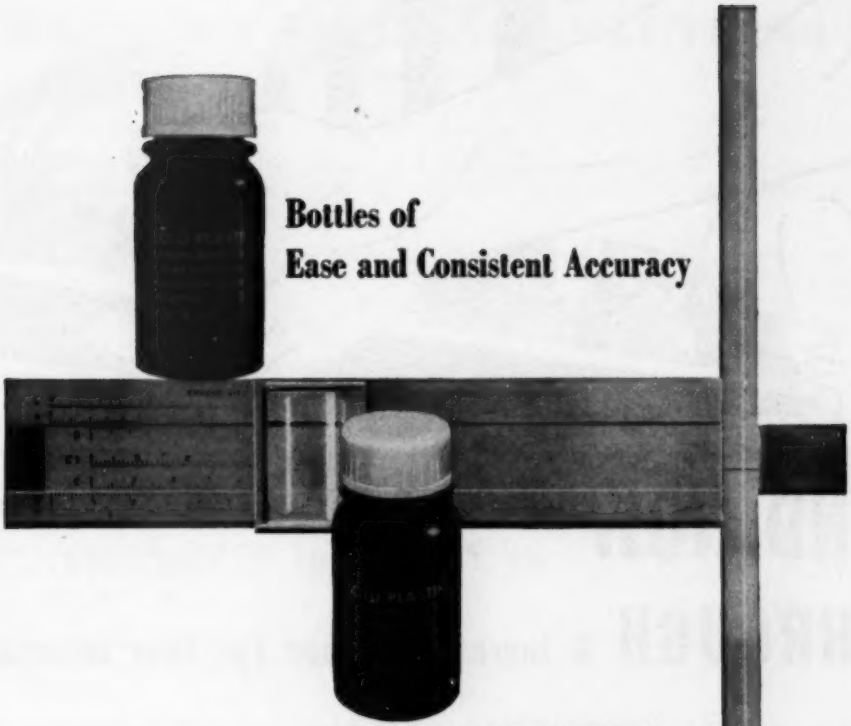
Progress in Biophysics and Biophysical Chemistry. Volume 2. Edited by J. A. V. Butler, Chester Beatty Research Institute, Royal Cancer Hospital, London, and J. T. Randall, F.R.S., Wheatstone Professor of Physics in the University of London at King's College. Price, $8.00. Pp. 323, with numerous illustrations. Academic Press, Inc., 125 E. 23rd St., New York 10, 1951.

These several reviews cover a larger range of topics at a more advanced theoretical and technical level than the ordinary reader might want to digest *in toto*. The special function of the biophysicist, we are told by one of the writers, is to expedite the application of recent advances in the physical sciences to biological problems. This clearly embraces most things in heaven and earth, and a bit more besides, it seems, since Mephistopheles is quoted (approvingly) at one point. It need hardly be added that a competent roster of contributors has been assembled.

Closest, perhaps, to the immediate interests of pathologists are two reviews covering biological reactions to radioisotopes and to ionizing radiations. The question of unitage and dosage are dealt with in some detail. A chapter on the quantum physics of vision refers to the extraordinary efficiency of the human eye as a light detector—so much so, that only a few quanta are necessary to excite. This brings its own problems, since fluctuations in the source become a significant factor in light perception.

Several articles deal with specialized applications of techniques; of birefringence measurements to cell membranes and cytoplasm; of x-ray diffraction and polarization microscopy to plant walls; of crystallography and x-rays to nucleic acids and their breakdown products; of electron microscopy to bacteriophage.

The most extensive treatment is a review of biophysical aspects of nervous function, heavily documented and presenting the findings of the great modern experimental and theoretical schools. Single neurons are first dealt with, then the properties of neurons en masse that cannot be extrapolated in any simple way from the former. This is salutary reading for those having inferiority feelings in the presence of giant computers and thinking machines. At least, we are assured that the latter have not yet got around to telling each other what to do.



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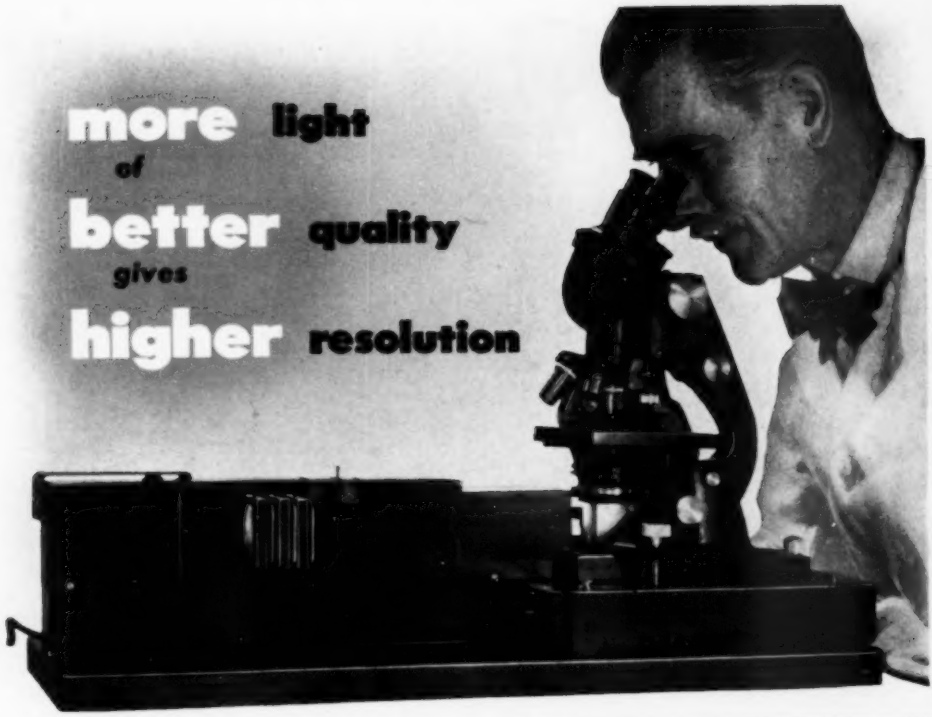

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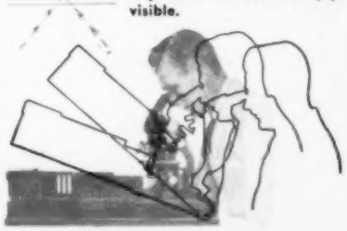

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